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(54) Title: COMPOSITIONS FOR CELL ADHESION INHIBITION AND METHODS OF USE

(57) Abstract

Compositions that disrupt microvascular endothelial and epithelial cell tight junctions, and methods of use, are disclosed. Such compositions comprise agents that inhibit the binding to such cells of cell adhesion molecules. Such inhibitor agents include cell adhesion molecules, fragments of cell adhesion molecules that encompass a cell-binding domain such as HAV, and antibodies directed against cell adhesion molecules and fragments thereof. Also disclosed are drug delivery compositions comprising a therapeutic drug conjugated to an agent that disrupts cell tight junctions.

DESIGNATIONS OF "DE"

Until further notice, any designation of "DE" in any international application whose international filing date is prior to October 3, 1990, shall have effect in the territory of the Federal Republic of Germany with the exception of the territory of the former German Democratic Republic.

COMPOSITIONS FOR CELL ADHESION INHIBITION
AND METHODS OF USE

This is a continuation-in-part of United States
Serial No. 07/413,332, filed September 27, 1989.

5   **Background of the Invention**
Field of the Invention
           This invention relates to compositions that
transiently and reversibly dissociate the blood-brain
barrier. More particularly, the invention relates to
10   compositions that dissociate tight junctions between
brain capillary endothelial cells that constitute the
physiological barrier between the general circulation
and the brain.

Detailed Description of Related Art
15   The entry of drugs from the blood stream to the
central nervous system (CNS), i.e., the brain and
spinal cord, is restricted by the presence of high
resistance tight junctions between brain capillary
cells and by the apparently low rate of transport
20   across these endothelial cells (Betz, A.L., et al.,
Ann. Rev. Physiol., 48:241 (1986); Pardridge, W.M.,
Ann. Rev. Pharmacol. Toxicol., 28:25 (1988)).
           The tight junctions of the blood brain barrier
(BBB) prevent diffusion of molecules and ions around
25   the brain capillary endothelial cells. The only
substances that can readily pass from the luminal core
of the capillary to the abluminal tissues that surround
the capillary are those molecules for which selective
transport systems exist in the endothelial cells, as
30   well as those compounds that are lipophilic (i.e.,
hydrophobic). In contrast, drugs, peptides and other

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molecules that are neither lipophilic nor transported
by specific carrier proteins are barred from entry into
the brain, or their rates of entry are too low to be
useful, thereby imposing a severe limitation upon the
5   physician's ability to treat CNS disorders
pharmacologically.

       The carrier-mediated transcellular transport
system mentioned above may have limited usefulness for
therapeutic modalities under some circumstances.
10   Transcytotic transport, in general, involves, first,
the binding of molecules to specific carrier proteins
on the surface of endothelial cells, and, second, the
delivery of such molecules across the endothelial
cells. Limitations on the usefulness of such a system
15   for treatment of CNS disorders are based on the
following considerations: (1) physiological carrier
proteins may not function efficiently, or at all, with
non-physiological drugs; (2) even where function
occurs, the rate of transport of therapeutic agents
20   will be limited by the rate of transport of the
carrier; (3) the overall capacity of cerebral capillary
endothelial cells to transport any therapeutic
macromolecules may be simply too low to achieve
therapeutic levels of certain drugs in the brain; and
25   (4) once therapeutic macromolecules enter endothelial
cells, depending on their nature, they might be
delivered to any number of organelles, including
lysosomes that contain a wide variety of hydrolytic
enzymes. For these reasons, creating drug delivery
30   systems that do not rely upon transcytosis will clearly
be advantageous.

       As tight junctions between brain capillary
endothelial cells constitute a major part of the BBB,
the possibility of modifying these junctions has been
35   considered. It has been found that tight junctions,

including those of the BBB, can be disrupted by
hyperosmotic solutions administered intra-arterially.
For example, Polley et al., WO89/04663, published
June 1, 1989, disclose the osmotic disruption of the

5   interendothelial structure of the BBB by the intra-
arterial administration of hypertonic solutions of
mannitol, arabinose or glycerol as a means of
introducing into the brain genetic material.
Similarly, hyperosmotic solutions of urea have also

10   been used to alter the BBB (Bowman, P.D. et al., Ped.
Res., 16:335A (1982)).

     Other chemical agents have been reported to
disrupt endothelial or epithelial cell tight junctions
when administered intravenously, including:

15   7-fluorouracil (MacDonell, L.A., et al., Cancer. Res.,
38:2930 (1978)), degradation by membrane enzymes
(Vincent, P.A., et al., Exp. Mol. Path., 48:403 (1988);
Diener, H.M., et al., J. Immunol., 135:537 (1985)),
aluminum salts (Zigler, Z.Y., et al., IRCS Med. Sci.,

20   12:1095 (1984)), histamine (Meyrick, B., et al., Exp.
Lung Res., 6:11 (1984)), thrombin (Siflinger-Birnboin,
A., et al., Microvasc. Res., 36:216 (1988)), phorbol
esters (Shiba, K., et al., Exp. Cell Res., 178:233
(1988)), and neutralization of the luminal anionic

25   charge (Hart, M.M., J. Neuropathol. Exp. Neurol.,
46:141 (1987)).

     Although the above-listed modalities may disrupt
tight junctions and thereby increase permeability of
the BBB, problems attendant upon their use make them

30   less than desireable. For example, intra-arterial
perfusion with hyperosmotic solutions involves surgery,
and this cannot be repeated on a regular basis.
Further, concentrated sugar solutions may not be
innocuous, and might be expected to have undesirable

35   side effects. In addition, the aforementioned chemical

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agents may not be useful for the treatment of chronic
neurological disease, their effects on tight junctions
are not always reversible, and, as they all are
themselves powerful drugs, there is always the danger
5   that their use will compromise the patient's health
generally. For example, 7-fluorouracil is a powerful
inhibitor of pyrimidine synthesis, and thus nucleic
acid biosynthesis, in animals cells.

Thus, an important need still exists for means
10   which transiently and reversibly disrupt tight
junctions of the BBB in order that administered drugs
can reach the brain from the general circulation, and
which have no undesirable side effects of their own in
the subject.

15   Attempts have been made to disrupt cell-cell
adhesion by modifying the protein(s) responsible for
such adhesion, collectively referred to as "cell
adhesion molecules" (CAM). One class of CAM is termed
"cadherin". "Cadherin" is the term applied to a family
20   of glycoproteins found in most kinds of mammalian
tissues and thought to be responsible for Ca^{2+}-
dependent cell-cell adhesion, (Takeichi, M.,
Development, 102:639 (1988)). Three subclasses of
cadherin have been identified, namely, E-cadherin (from
25   epithelial tissues), P-cadherin (from placental
tissues), and N-cadherin (from neural tissues)
(Yoshida-Noro, C., et al. Dev. Biol., 101:19 (1984);
Nose, A., et al., J. Cell Biol., 103:2649 (1986);
Hatta, K., et al., Nature, 320:447 (1986)).

30   The different cadherins exhibit distinct tissue
distribution patterns (Takeichi, U., (1988) above).
E-cadherin, which was found to be distributed
exclusively in epithelial cells of various tissues
(Hatta, K., et al., Proc. Nat'l. Acad. Sci. (USA),
35   82:2789 (1985); Takeichi, 1988, above), appears to be

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identical to uvomorulin (Hyafil, F., et al., Cell, 21:927 (1986)), chicken liver-cell adhesion molecule (L-CAM, Gallin, W.J., et al., Proc. Nat. Acad. Sci. (USA), 80:1038 (1983)), and cell-CAM 120/80 (Damsky,
5   C.H., et al., Cell, 34:455 (1983)) in terms of biochemical properties (Cunningham, B.A., et al., Proc. Nat. Acad. Sci. (USA), 81:5787 (1984)) and tissue distributions (Thiery, J.-P., et al., Dev. Biol., 102:61 (1984)).

10   N-cadherin, which is expressed in various neural tissues including astrocytes (Hatta, K., et al., Devel. Biol., 120:215 (1987); Matsunega, M., et al., Nature, 334:62 (1988); Tomaselli, K.J., Neuron, 1:33 (1988)), shows 92% amino acid sequence homology between
15 mammalian and avian homologs, shows from 40 to 50% similarity to epithelial E-cadherin and to placental P-cadherin of the same species, but was immunologically not cross-reactive with other cadherins within the same animal (Miyatani, S., Science, 245:631 (1989)).

20   Placental P-cadherin has also been cloned, and the deduced amino acid sequence of this glycoprotein was found to exhibit about 58% homology with epithelial E-cadherin (Nose, A., et al., EMBO J., 12:3655 (1987)).

        Subsequent to the September 27, 1989 filing of the
25 parent application, Heimark, et al. (Heimark, R.L., et al., J. Cell Biol., 110:1745 (1990) reported on the identification of a Ca^{2+}-dependent cell-cell adhesion molecule in aortic endothelial cells.

        Although each of the aforelisted cadherins
30 displays unique immunological and tissue distribution specifications, all have features in common: (1) a requirement for Ca^{2+} for cell adhesion function; (2) protection by Ca^{2+} from proteolytic cleavage; (3) similar numbers of amino acids, i.e., from about 723 to
35 about 822; (4) similar masses, i.e., about 124 kdal.

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for the glycoprotein; (5) substantial interspecies
(50%-60%) overall sequence homology with interspecies
homologies increasing to about 56% to 99% in the
cytoplasmic region of the protein, suggesting that they
5   constitute a gene family (Nose, A., 1987; Miysysni, D.,
et al., 1989); and (6) a common mechanism of action,
namely, homophilic binding of cadherins on one cell to
similar cadherins on the adjoining cell.

CAMs independent of Ca^{2+} are also known, for
10 example, the 125K glycoprotein of Urushihara et al.
(Urushihara, H., et al., Cell, 20:363 (1980)); N-CAM
(Rutishauser, U., Nature, Lond., 310:549 (1984));
Ng-CAM (Grunet, M. et al., Proc. Nat'l. Acad. Sci.
(USA), 81:7989 (1984)); Ll (Rathjien, F.G. et al., **XXXXXXXXX
15 J., 3:1 (1984)); G4 (Rathjien, F.G. et al., J. Cell
Biol., 104:343 (1987)); and platelet glycoprotein
PECAM-1 (CD 31) (Newman, P.J., Science, 247:1219
(1990)). Ca^{2+}-independent CAMs are known to exhibit
certain properties of the Ca^{2+}-dependent CAMs. Thus,
20 N-CAM and N-cadherin both promote retinal neurite
outgrowth on astrocytes (Neugebauer, K.M., et al., J.
Cell Biol., 107:1177 (1985)), and on Schwann cells
(Bixby, J.L. et al., J. Cell Biol., 107:353 (1988)).

Monoclonal antibodies raised against epithelial
25 E-type cadherins such as uvomorulin are known to
disrupt the adhesion of several cell types, including
embryo cells, cultured teratocarcinoma cells,
hepatocytes, and MDCK kidney epithelial cells (Ogou,
S.-I., et al., J. Cell Biol., 97:944 (1983); Yoshida-
30 Noro, et al., (1984), above; Shirayoshi, Y., et al.,
Cell Struct. Funct., 11:285 (1986); Gallin, et al.,
(1983), above; Vestweber, D., et al., EMBO J., 4:3393
(1985); Johnson, M.H., et al., J. Embrol. Exp.
Morphol., 93:239 (1986); Gumbiner, B., et al., J. Cell
35 Biol., 102:457 (1986)).

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However, prior to the present discoveries disclosed in the parent applications cadherins had not been found in brain capillary or other endothelial cells (see, Takeichi, et al. (1988), above). Further, the CAMs of microvascular endothelial cells had not yet been identified, nor had such molecules been localized specifically to brain capillary endothelial cells. Thus, until the present invention no means were known for transiently and reversibly disrupting tight junctions between microvascular endothelial cells, including those of the BBB, based upon an attack upon the CAM's of such cells that are responsible for tight junction formation and maintenance.

It has been hypothesized that the cadherins contain a common cell adhesion recognition (CAR) sequence. The CAR sequences of several cell and substratum adhesion molecules are known. Martin, G.R., et al., Ann. Rev. Cell Biol., 3:57 (1987) ; Ruoslahti, E., et al., Science, 238:491 (1987). In general, CAR sequences are composed of at least three amino acid residues. The most rigorously investigated CAR sequence is RGD which is found in laminin, fribronectin and other basement membrane components that are responsible for the binding of cells to the substratum.

Blaschuk, et al., in a paper to be published subsequent to the filing of the present application (Blaschuk, O., et al., J. Mol. Biol., in press, (1990)), disclose the presence of three potential cadherin CAR sequences in the first extracellular domains of liver CAM, E-, P-, and N-cadherin, namely, PPI, GAD and HAV. Blaschuk, et al. (Blaschuk, O., et al., Develop. Biol., 139:227 (1990)), also disclosed recently that synthetic peptides containing the HAV sequence inhibited two biological processes (compaction of 8-cell-stage mouse embryos and rate of neurite

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outgrowth on astrocytes) that are known to be mediated
by cadherins. Effective peptides in these assays were
LRAHAVDVNG and AHAVSE; PPI-containing peptides were
without effect. However, Blaschuk et al. provide no
5   guidance for determining the regions flanking the HAV
tripeptide that are critical for cell-cell adhesion.
In the BBB disrupting peptides of the present invention
detailed below, we have observed that the mere presence
of the HAV sequence in a small cadherin-derived peptide
10 is not the sine qua non for a composition effective to
prevent cell-cell adhesion. Indeed, it should be
emphasized that neither Blaschuk et al. nor any other
publication known to the present inventors suggest that
cadherin sequences containing HAV or SHAVS sequences
15 would be effective in opening tight junctions and
piercing blood brain barriers formed by E-cadherins in
brain microvascular endothelial cells.

SUMMARY OF THE INVENTION

It has now been discovered that molecules
20 homologous to, and immunologically related to, cadherin
cell adhesion molecules are present on brain and non-
brain microvascular endothelial cells, such that

junctions between such endothelial cells can be reversibly opened so as to permit passage of therapeutic drugs by the use of polypeptide and antibody compositions that compete with such cell
5   adhesion molecules for binding to such cells.

It is therefore an object of this invention to provide the identity of microvascular endothelial cell adhesion molecules.

Another object of this invention is to provide DNA
10 sequences of genes, and plasmids containing same, coding for the expression of all or a cell-binding portion of microvascular endothelial cell adhesion molecules.

Yet another object of this invention is to provide
15 means to identify those sequences of cell adhesion molecules responsible for the tight binding of adjoining endothelial cells.

A further object is to provide therapeutic compositions comprising polypeptides derived from cell
20 adhesion molecules that reversibly disrupt cell-cell adhesion.

Still another object of this invention is to provide therapeutic compositions comprising polyclonal or monoclonal antibodies or fragments thereof directed
25 against endothelial cell adhesion molecules, or against polypeptides representing cell binding regions thereof, that reversibly disrupt endothelial cell-cell adhesion.

Yet another object of this invention is to provide therapeutic formulations comprising therapeutic drugs
30 conjugated with blood-brain barrier-disrupting compositions of this invention, that are capable of entering the central nervous system following disruption of the blood-brain barrier.

These and other objects of this invention will
35 become clear by reference to the following description

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of the invention and to the appended claims.

DESCRIPTION OF THE DRAWINGS

Figure 1 illustrates the partial cDNA sequence for bovine endothelial cell adhesion molecule homologous to chicken N-cadherin.

Figure 2 illustrates the partial cDNA sequence for bovine endothelial cell adhesion molecule homologous to mouse P-cadherin.

Figure 3 illustrates the cDNA sequence for the MDCK cell adhesion molecule homologous to mouse E-cadherin.

Figure 4 illustrates the restriction sites in the bovine endothelial cell N- (4-1 to 4-5) and P-cadherin (4-6 to 4-8) cDNA sequences and in the MDCK E-cadherin (4-9 to 4-14) cDNA sequence.

Figure 5 shows the staining of a mouse brain thin section by an antibody raised against a fusion protein derived from amino acids 9-96 of MDCK E-cadherin containing an HAV region.

Figure 6 is a repeat of the experiment of Fig. 5, except that the antibody was raised against the entire E-cadherin molecule.

Figure 7 illustrates the effects of an 18-mer HAV-containing polypeptide on the resistance of tight junction monolayers of MDCK epithelial cells.

Figure 8 illustrates the effects of 11-mer and 18-mer HAV-containing polypeptides on the resistance of tight junction monolayers MDCK epithelial cells.

Figure 9 illustrates the effects of 11-mer and 18-mer HAV-containing polypeptides on the resistance of tight-junction monolayers of brain microvascular endothelial cells.

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DETAILED DESCRIPTION OF THE INVENTION

It has now been discovered that cell adhesion molecules with characteristics of cadherins are present on the surfaces of brain capillary endothelial cells

5   and of microvascular endothelial cells of non-brain origins. The present invention is based on the discovery that a polypeptide composition comprising cell binding domains of endothelial cell adhesion molecules may compete against such molecules for

10 binding to such cells, such that by this means the junctions between such cells could be reversibly opened, thereby permitting penetration by therapeutic agents. The present invention also discloses that polyclonal or monoclonal antibodies (or fragments

15 thereof) raised against endothelial cell adhesion molecules or cell-binding domains thereof may also compete for endothelial cell surface binding sites, and, by this means, reversibly disrupt junctions between endothelial cells, thereby permitting entry

20 into the central nervous system of therapeutic agents.

In order to obtain compositions useful for disrupting tight junctions between microvascular endothelial cells, the cell adhesion molecules responsible for such junctions were identified.

25   The endothelial cell cadherins disclosed herein exhibit one or more of several characteristics of E-, P- and N- cadherins, including: characteristics of a transmembrane integral protein, with cytoplasmic, hydrophobic plasma membrane, and extracellular regions;

30 intraspecies DNA sequence homologies of greater than about 50% for the entire molecule; immunological cross-reactivity with antibodies raised against non-endothelial cell cadherins; and containing cell-binding domains. "Immunologically related to" means that these

35 cadherin-like molecules cross-react with antibodies

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raised against non-endothelial cell cadherins.

E-cadherin-like molecules were localized in brain by immunofluorescence. Cryostat sections of mouse brain were labeled with a rabbit antibody prepared
5 against E-cadherin, and then with fluorescein isothiocyanate-conjugated goat anti-rabbit immunoglobulin. There is clear labeling of a capillary in brain sections as shown by immunofluorescence microscopy. Endothelial cells in liver and kidney were
10 not stained by this procedure.

cDNAs coding for the expression of bovine microvascular endothelial cell (BMEC) cadherins were cloned and sequenced as described below, and the partial sequence of N-cadherin and P-cadherin are
15 disclosed herein in Figures 1 and 2, respectively. In addition, as MDCK dog kidney epithelial cells are known to employ E-cadherin to form high resistance tight junctions, and as the present invention discloses that brain capillary endothelial cell adhesion molecules
20 include E-type cadherin, the DNA of this cadherin was also cloned; its complete DNA sequence is disclosed herein (Fig. 3).

N-, P- and E-cadherin-type clones described herein were deposited in the American Type Culture Collection
25 on September 26, 1989, and were assigned the following accession numbers:

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Clone Designation	Accession No.

N-cadherin-type clones
     pUC19-bNCad   10A   40667
     pUC19-bNCad 39A   40669

5   P-cadherin-type clones
     pUC18-bPCad   3B-10   40668
     pUC19-bPCad   9B   40670

     E-cadherin-type clones
     pBluescript MDCKECad 45-30E   40671

10   The cloning of cadherins was accomplished by taking advantage of the fact that the cadherins characterized thus far are transmembrane glycoproteins, the cytoplasmic domains of which are highly conserved, that is, are highly homologous.

15   Two degenerate oligonucleotides flanking the 42-amino acid coding region in the cytoplasmic domain were selected to serve as primers for polymerase chain reaction (PCR) using either BMEC cDNA or MDCK cDNA as templates. The PCR reactions were carried out

20 essentially according to Saiki, R. K. et al., Science, 239:487 (1988), which is incorporated herein by reference.

     The cloned PCR products from each cell type were sequenced essentially according to the method of

25 Sanger, F. et al., Proc. Nat'l. Acad. Sci. (USA), 74:5463 (1977), which is incorporated herein by reference.

     It was discovered that BMEC cadherins are of two types - one homologous to chicken N-cadherin (neuronal

30 type, see, e.g., Hatta, K., et al., J. Cell Biol., 106:873 (1988)) and the other homologous to mouse P-cadherin (placental type, see e.g., Nose, A., et al., (1987) above). It has also been found that there are two species of cadherins in MDCK cells - one homologous

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to mouse E-cadherin (see, e.g., Nagafuchi, A., et al.,
Nature, 329:341 (1987)) and the other homologous to
mouse P-cadherin (Nose, et al. (1987), above).

5   The PCR products were then used as probes to
isolate the BMEC and MDCK cadherin cDNA clones as
follows. A cDNA library was constructed essentially
according to Gubler et al. (Gubler, U. et al., Gene,
25:263 (1983), which is incorporated herein by
reference), using poly (A)$^+$RNA isolated from either
10   BMEC or MDCK cells. The cDNA was ligated via EcoRI
adaptors into gt10 arms (BMEC) or ZAP^R (from
Stratagene, Inc., La Jolla, CA) vector arms (MDCK).
cDNA libraries containing 5 x 10^5 - 1.5 x 10^6
independent cDNA clones were screened using
15   radiolabeled PCR products (Benton, W.D. et al.,
Science, 196:180 (1987), which is incorporated herein
by reference). Northern blot analysis (Maniatis, T. et
al., "Molecular Cloning: A Laboratory Manual", Cold
Spring Harbor Laboratory, Cold Spring Harbor, N.Y.,
20   1982) may be used to determine whether each cDNA
species cloned hybridizes to a single mRNA species, as
well as the tissue distributions of each cDNA species.
     cDNA clones for each cadherin were sequenced by
the method of Sanger et al. (1977) above.
25   The partial restriction maps for each cDNA clone
based on their sequences are shown in Fig. 4. Some of
these restriction sites were confirmed by restriction
enzyme digestions, including Hind III, Pst I, Kpn I,
Bgl II for N-cadherin; Pvu II, Sac I and Pst I for
30   P-cadherin; Pst I, Pvu II, BamH I, and Sac I for
E-cadherin.
     In order to test whether the cloned E-cadherin
cDNA contains all the information necessary for
cadherin function, full-length E-cadherin cDNA joined
35   to a suitable promoter may be introduced into mouse

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L-cells that have very little endogenous cadherin
activity (Nagafuchi, et al. (1987), supra). To test
for expression of E-cadherin in transfectants derived
from the introduced cDNA, transfected L-cells may be
5   tested for Ca^{2+}-dependent aggregating activity. The
extent of this aggregating activity should be closely
correlated with the amount of E-cadherin expressed
(Takeichi, M. (1988), supra). This same technique may
be used for testing cDNAs encoding bovine endothelial
10   N- and P-cadherins, according to the method of Hatta,
et al. (Hatta, K., et al. (1988), supra).

In order to identify cell binding domains in, for
example, MDCK E-type cadherin, L-cells may be first
transfected as above with a cDNA of a size sufficient
15   to cause Ca^{2+}-mediated aggregation of transfectants. A
series of deletion mutants comprising truncated cDNA
species missing different regions of the extracellular
domain may be prepared by restriction enzyme digestion
and proper end filling or exonuclease digestion to make
20   the deletions in the proper coding frames. These
deletion mutants can then be tested for their ability
to express in L-cells a protein causing Ca^{2+}-dependent
aggregation. By correlating a loss of aggregation with
deletion of particular fragments, the regions important
25   for cell binding may be determined. A variety of
polypeptides corresponding to binding regions of
cadherins, as deduced from the nucleotide sequences of
deleted cDNA, may be synthesized chemically using an
automated peptide synthesizer such as that of Applied
30   Biosystems, Inc., Foster City, CA, or expressed by
recombinant DNA methods. Effective polypeptides may be
of varying lengths, depending upon the natures of
junctions being disrupted and the cell adhesion
molecule present.

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Nucleotide, and corresponding amino acid,
sequences of cadherins may be analyzed to detect
homologous regions. Applying this technique to bovine
endothelial cell N- and P-cadherins and to epithelial
5   cell E-cadherin, we have determined that, in the amino
acid 80 region of each of these cadherins, there is
conserved a triplet HAV (His-Ala-Val) region. We have
deduced that this HAV region may be a common cell
adhesions recognition (CAR) sequence.

10   We have chemically synthesized the following
polypeptides, each of which containing the HAV
sequence:

      6-mer(78-83) NH_2-SHAVSS-$CONH_2$
      11-mer(76-86) NH_2-LYSHAVSSNGN-$CONH_2$
15   17-mer(74-90) NH_2-YILYSHAVSSNGNAVED-$CONH_2$
      18 mer(69-86) NH_2-EQIAKYILYSHAVSSNGN-$CONH_2$
      20-mer(71-90) NH_2-IAKYILYSHAVSSNGNAVED-$CONH_2$

and have tested each for efficacy in opening brain
endothelial cell tight junctions in the BBB model
20   disclosed in copending United States application Serial
No. 07/413,274, and also on kidney epithelial cell
tight jucntions..

      Polyclonal antibodies raised in rabbits and
monoclonal antibodies derived from hybridomas may be
25   generated against each of the chemically-synthesized
polypeptides by standard methods. (Harlow, E., et al.,
"Antibodies: A Laboratory Manual", Cold Spring Harbor
Laboratory Press, Cold Spring Harbor, N.Y., 1988;
Goding, J.W., "Monoclonal Antibodies: Principles and
30   Practice", Academic Press, N.Y. 1986). In addition,
recombinant antibodies may be prepared. Fragments of
antibodies, e.g., Fc, Fab, F(ab)', may be prepared by
standard methods.

      We have cloned and sequenced fusion proteins
35   derived from amino acids 9-96 of MDCK E-cadherin

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containing the HAV region. A polyclonal antibody prepared against this fusion protein stained rat (Fig.55) mouse brain sections as well as did an antibody raised against the entire E-cadherin (Fig. 6).

5   A polyclonal antibody raised against a fusion protein derived from amino acids 9-37 failed to stain brain sections. These results indicate that the key cell-binding domain of E-cadherin lies in the region of amino acids 37-96.

10   The ability of CAM-derived polypeptides containing cell-binding domains, and the corresponding polyclonal and monoclonal antibodies, of the invention to disrupt tight junctions may be tested in in vitro and in vivo models of high resistance tight junctions and in animal

15   models. Monolayers of MDCK dog kidney epithelial cells, that are known to contain high resistance tight junctions (Gumbiner, B., J. Cell Biol., 102:457 (1986)), can be used to test for the ability of the polypeptides and corresponding antibodies of the

20   present invention to disrupt such tight junctions.

         Polyclonal antibodies prepared as described above may also be used in conjunction with Western blotting (Old, R.W., et al., Principles of Gene Manipulation, 3d ed., Blackwell, Oxford, 1985, p. 10) and a variety of

25   tissue extracts in order to identify cell adhesion glycoproteins in such extracts.

         Another embodiment of the present invention is in drug delivery systems. Conjugates between therapeutic drugs and agents that affect cell adhesion molecule

30   function in brain capillary endothelial cells may be used to deliver therapeutic drugs to the CNS. For example, a polypeptide derived from a cell adhesion molecule that contains within its amino acid sequence a cell-binding domain, or antibodies thereto, may be

35   conjugated in biologically-active form to a therapeutic

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modality. Such conjugates may have the dual effect of
opening the BBB and delivering the therapeutic agent to
the brain side of the BBB. Delivery of therapeutic
drugs to the CNS, either alone or conjugated to agents
5   that disrupt cell-cell adhesion, may be accomplished by
administering such drugs to a subject either
simultaneously with or subsequent to the administration
of the agents of this invention that disrupt the tight
junctions of the BBB. Examples of therapeutic
10   modalities that may be delivered to the brain by the
cell adhesion disruption compositions of this invention
include Nerve Growth Factor, anti-Parkinsonian drugs,
and brain enzymes known to be missing in
sphingolipidoses, e.g., Tay-Sachs disease. Means of
15   chemically conjugating protein or polypeptide carriers
to therapeutic agents such that the biological
integrity of the therapeutic agent is not compromised
and such that the therapeutic agent is readily cleaved
from the carrier by enzymes present on or within
20   endothelial cells (e.g., amidases, esterases,
disulfide-cleaving enzymes), are well known in the art.
It is also apparent that these therapeutic conjugates
may be delivered to endothelial cells in encapsulated
form (e.g., in liposomes) or as microsuspensions
25   stabilized by pharmacological excipients.

It is known (Jain, R.K., _J. Natn'l Cancer Inst._,
81:570 (1989)) that many solid tumors develop internal
barriers, including high pressure zones and collapsed
blood vessels, that make it difficult for blood-borne
30   chemotherapeutic agents to reach the tumor's inner
core. The barrier problem is particularly troublesome
with therapeutic products drawn from the human immune
system, such as monoclonal antibodies conjugated with
chemotherapeutic agents, interleukin-2, interferon and
35   activated killer T-lymphocytes, because of their large

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size. Thus, in another embodiment of this invention,
compositions that disrupt the junctions between
endothelial cells, particularly the relatively small
peptides that contain one or more cell-binding regions
5   of cell adhesion macromolecules, may be used to enhance
drug delivery to tumors with depressed blood flow.

It has been theorized that cancer cells
metastasize by secreting soluble cadherins variously to
open tight junctions in cells that block their movement
10 and to prevent their being bound to such cells. We
consider it likely that antibodies raised against these
cadherins, which are derived from extracellular domains
of the cadherins disclosed in this invention, may
provide a therapeutic modality that inhibits or
15 prevents cancer cell metastases.

In another embodiment, the compositions of this
invention may also be used to provide penetration for
chemotherapeutic agents of other well-known blood-
tissue barriers, such as blood-testis barriers and
20 blood-retina barriers. The latter barrier is known to
prevent the efficient transport of, for example,
administered antibiotics to the retina from the general
circulation. The cell adhesion disrupting compositions
of this invention may, thus, be used in conjunction
25 with the administration of antibiotics to treat retinal
infections.

The following examples are illustrative of several
embodiments of this invention, and should not be
construed in any way as limiting the invention as
30 recited in the claims.

EXAMPLE 1

EFFECTS OF HAV-CONTAINING POLYPEPTIDES
ON TIGHT JUNCTIONS OF MDCK EPITHELIAL
AND BOVINE ENDOTHELIAL CELLS

20

The BBB model of copending U.S. Serial No.
07/413,332 was used to examine the effects of
polypeptides containing the HAV region on the tight
junctions of monolayers of MDCK epithelial cells and
5   bovine capillary endothelial cells as determined by
resistance measurements across the monolayers.

The polypeptide was added to the cells either from
the apical side (top) or basolateral side (bottom), as
shown in the following sketch.

10   APICAL

EPITHELIAL CELLS   ENDOTHELIAL CELLS
   Gut Side   Blood Side

   Blood Side   Brain Side

                        BASOLATERAL

15   Figure 7 illustrates the effects of various
concentrations of the aforementioned 18-mer polypeptide
on resistance of MDCK epithelial cells. At the lowest
concentration tested, 0.5 mg/ml, resistance was
markedly decreased. The polypeptide was more effective
20   when added from the basolateral side, but at high
concentrations was quite effective even when added from
the apical side. These data indicate that the 18-mer
is effective in making tight junctions permeable. The
20-mer was similarly effective, and a 17-mer less
25   effective.

Figure 8 illustrates the effects of the
aforementioned 11-mer and 18-mer on MDCK cell
resistance when added from either the apical or
basolateral side of the monolayers. The concentration
30   of polypeptide was about 1 mg/ml. The 11-mer (as well

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as the 6-mer data not shown) was virtually without effect. With the 18-mer, resistance was almost totally abolished by about 6 hours, indicating disruption of tight junctions. That the effect of the 18-mer is

5   reversible is indicated by the "wash-out" experiment. When the 18-mer was washed out of the MDCK cells at 6 hours, resistance recovered to a substantial extent over the next 21 hours. This recovery was particularly pronounced when the 18-mer had originally been added

10   from the basolateral side of the monolayers. The 20-mer produced results similar to those of the 18-mer, and the 17-mer was effective, but somewhat less so.

Figure 9 illustrates the effect of 1 mg/ml of the 11-mer and 18-mer on high resistance monolayer cultures

15   of brain endothelial cells (see copending United States Serial No. 07/413,332 for method of preparation). As with MDCK cells, the 11-mer (and the 6-mer) failed to reduce resistance values over a 48-hour period of observation. In contrast, the 18-mer (as well as the

20   20-mer) decreased resistance values markedly when added from either the basolateral or apical side, but the effect of the polypeptide was more rapid and more pronounced when it was added from the basolateral side; the 17-mer was less effective.

25   The conclusion of these experiments is that a particular set of peptides (but not all peptides) centered around the HAV region of E-cadherin are effective in opening tight junctions of brain endothelial cell blood-brain barriers, and also of

30   epithelial cells that form such junctions ("gut barrier"). Both the length and compositon of the amino acid region flanking the HAV triplet thus appear to play a role in the efficacy of such compositions.

While the aforementioned embodiments represent the

35   preferred embodiments of the invention, those skilled

22

in the art may, without undue experimentation, devise
other executions of the compositions and methods of use
of this invention without departing from the concept
and spirit inherent therein.

23

What is claimed is:

1. A composition for opening tight junctions between microvascular endothelial cells of a subject, whereby means are provided for a drug to cross the permeability barrier imposed by such junctions, comprising an agent capable of reacting with at least one type of cell-bound cell adhesion molecule that would otherwise mediate tight junction formation between microvascular endothelial cells, so that cell-cell adhesion is disrupted.

2. A composition of claim 1, wherein said cell adhesion molecule exhibits at least about 50% sequence homology with a cadherin selected from the group consisting of E-cadherin, N-cadherin and P-cadherin.

3. A composition of claim 1, wherein said cell adhesion molecule is immunologically related to at least one of the group consisting of E-cadherin, N-cadherin and P-cadherin.

4. A composition of claim 1, wherein the microvascular endothelial cells are brain capillary endothelial cells.

5. A composition of claim 2, wherein said agent comprises an inhibitor of the binding to cells of said cell adhesion molecule.

6. A composition of claim 3, wherein said agent comprises an inhibitor of the binding to cells of said cell adhesion molecule.

7. A composition of claim 5, wherein said inhibitor agent comprises a fragment of said cell adhesion molecule.

8. A composition of claim 7, wherein said cell adhesion molecule fragment includes within its amino acid sequence a cell-binding domain.

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9.   A composition of claim 8, wherein said cell-binding domain contains an HAV amino acid sequence.

10.   A composition of claim 9, wherein said amino acid sequence is

$$NH_2-YILYSHAVSSNGNAVED-CONH_2 \qquad .$$

11.   A composition of claim 9, wherein said amino acid sequence is

$$NH2-EQIAKYILYSHAVSSNGN-COHN_2 \qquad .$$

12.   A composition of claim 9, wherein said amino acid sequence is

$$NH_2-IAKYILYSHAVSSNGNAVED-CONH_2 \qquad .$$

13.   A composition of claim 9, wherein said amino acid sequence comprises amino acids 9-96 of E-cadherin.

14.   A composition of claim 5, wherein said inhibitor agent comprises a polyclonal or monoclonal antibody directed against said cell adhesion molecule.

15.   A composition of claim 5, wherein said inhibitor agent comprises a polyclonal or monoclonal antibody directed against a fragment of said cell adhesion molecule.

16.   A composition of claim 15, wherein said cell adhesion molecule fragment includes within its amino acid sequence a cell-binding domain.

17.   A composition of claim 16, wherein said cell-binding domain contains an HAV amino acid sequence.

25

18.   A composition of claim 17, wherein said amino acid sequence is

$$NH_2-YILYSHAVSSNGNAVED-CONH_2$$.

19.   A composition of claim 17, wherein said amino acid sequence is

$$NH2-EQIAKYILYSHAVSSNGN-COHN_2$$.

20.   A composition of claim 17, wherein said amino acid sequence is

$$NH_2-IAKYILYSHAVSSNGNAVED-CONH_2$$.

21.   A composition of claim 17, wherein said amino acid sequence comprises amino acids 9-96 of E-cadherin.

22.   A composition of claim 5 or 6 in a pharmaceutically-acceptable vehicle.

23.   A method for opening tight junctions between microvascular endothelial cells of a subject, comprising the step of administering to the subject an agent, in an effective amount and in a

5   pharmaceutically-acceptable vehicle, capable of reacting with at least one type of cell-bound cell adhesion molecule that would otherwise mediate tight junction formation between microvascular endothelial cells, so that cell-cell adhesion is disrupted and

10   whereby means are provided for a drug to cross permeability barriers imposed by such tight junctions.

24.   A method of claim 23, wherein said cell adhesion molecule exhibits at least about 50% homology with a cadherin selected from the group consisting of E-cadherin, N-cadherin and P-cadherin.

25. A method of claim 23, wherein said cell adhesion molecule is immunologically related to at least one of the group consisting of E-cadherin, N-cadherin and P-cadherin.

26. A method of claim 23, wherein the microvascular endothelial cells are brain capillary endothelial cells.

27. A method of anyone of claims 23-25, inclusive, wherein said agent comprises an inhibitor of the binding to cells of said cell adhesion molecule.

28. A method of claim 27, wherein said inhibitor agent comprises a fragment of said cell adhesion molecule.

29. A method of claim 28, wherein said cell adhesion molecule fragment includes within its amino acid sequence a cell-binding domain.

30. A method of claim 29, wherein said cell-binding domain contains an HAV amino acid sequence.

31. A method of claim 30 wherein said amino acid sequence is

$$NH_2-YILYSHAVSSNGNAVED-CONH_2 \qquad .$$

32. A method of claim 30, wherein said amino acid sequence is

$$NH2-EQIAKYILYSHAVSSNGN-COHN_2 \qquad .$$

33. A method of claim 30, wherein said amino acid sequence is

$$NH_2-IAKYILYSHAVSSNGNAVED-CONH_2 \qquad .$$

34. A method of claim 30, wherein said amino acid sequence comprises amino acids 9-96 of E-cadherin.

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35. A method of claim 27, wherein said inhibitor agent comprises a polyclonal or monoclonal antibody directed against said cell adhesion molecule.

36. A method of claim 28, wherein said inhibitor agent comprises a polyclonal or monoclonal antibody directed against said fragment of said cell adhesion molecule.

37. A method of claim 36, wherein said cell adhesion fragment includes within its amino acid sequence a cell-binding domain.

38. A method of claim 37 wherein said cell-binding domain contains an HAV amino acid sequence.

39. A method of claim 38, wherein said amino acid sequence is

NH_2-YILYSHAVSSNGNAVED-$CONH_2$.

40. A method of claim 38, wherein said amino acid sequence is

NH2-EQIAKYILYSHAVSSNGN-$COHN_2$.

41. A method of claim 38, wherein said amino acid sequence is

NH_2-IAKYILYSHAVSSNGNAVED-$CONH_2$.
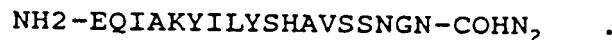
42. A method of claim 38, wherein said amino acid sequence comprises amino acids 9-96 of E-cadherin.

43. A drug delivery composition comprising a conjugate between a therapeutic drug and an agent capable of reacting with at least one type of a cell-bound cell adhesion molecule that would otherwise
5   mediate tight junction formation between microvascular endothelial cells, so that cell-cell adhesion is

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disrupted by said agent, whereby means are provided for
said drug to cross permeability barriers imposed by
such tight junctions, in a pharmaceutically-acceptable
10   vehicle.

44.   A drug delivery composition of claim 43,
wherein said cell adhesion molecule exhibits at least
about 50% homology with a cadherin selected from the
group consisting of E-cadherin, N-cadherin and
P-cadherin.

45.   A drug delivery composition of claim 43,
wherein said cell adhesion molecule is immunologically
related to at least one of the group consisting of
E-cadherin, N-cadherin and P-cadherin.

46.   A drug delivery composition of claim 43,
wherein the microvascular endothelial cells are brain
capillary endothelial cells.

47.   A drug delivery composition of any one
of claims 43-45, inclusive, wherein said agent
comprises an inhibitor of the binding to cells of said
cell adhesion molecule.

48.   A drug delivery composition of claim 47,
wherein said agent comprises a fragment of said cell
adhesion molecule.

49.   A drug delivery composition of claim 48,
wherein said cell adhesion molecule fragment includes
within its amino acid sequence a cell-binding domain.

50.   A drug delivery composition of claim 49,
wherein said cell-binding domain contains an HAV amino
acid sequence.

51.   A drug delivery composition of claim 50,
wherein said amino acid sequence is

NH_2-YILYSHAVSSNGNAVED-$CONH_2$.

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52.   A drug delivery composition of claim 50,
wherein said amino acid sequence is

        NH2-EQIAKYILYSHAVSSNGN-COHN$_2$.

        53.   A drug delivery composition of claim 50,
wherein said amino acid sequence is

        NH$_2$-IAKYILYSHAVSSNGNAVED-CONH$_2$.

        54.   A drug delivery composition of claim 50,
wherein said amino acid sequence comprises amino acids
9-96 of E-cadherin.
        55.   A drug delivery composition of claim 43,
wherein said inhibitor agent comprises a polyclonal or
monoclonal antibody directed against said cell adhesion
molecule.
        56.   A drug delivery composition of claim 43,
wherein said inhibitor agent comprises a polyclonal or
monoclonal antibody directed against a fragment of said
cell adhesion molecule.
        57.   A drug delivery composition of claim 56,
wherein said cell adhesion molecule fragment contains
within its amino acid sequence a cell-binding domain.
        58.   A drug delivery composition of claim 56,
wherein said cell-binding domain encompasses an HAV
amino acid sequence.
        59.   A drug delivery composition of claim 58,
wherein said amino acid sequence is

        NH$_2$-YILYSHAVSSNGNAVED-CONH$_2$.

        60.   A drug delivery composition of claim 58,
wherein said amino acid sequence is

30
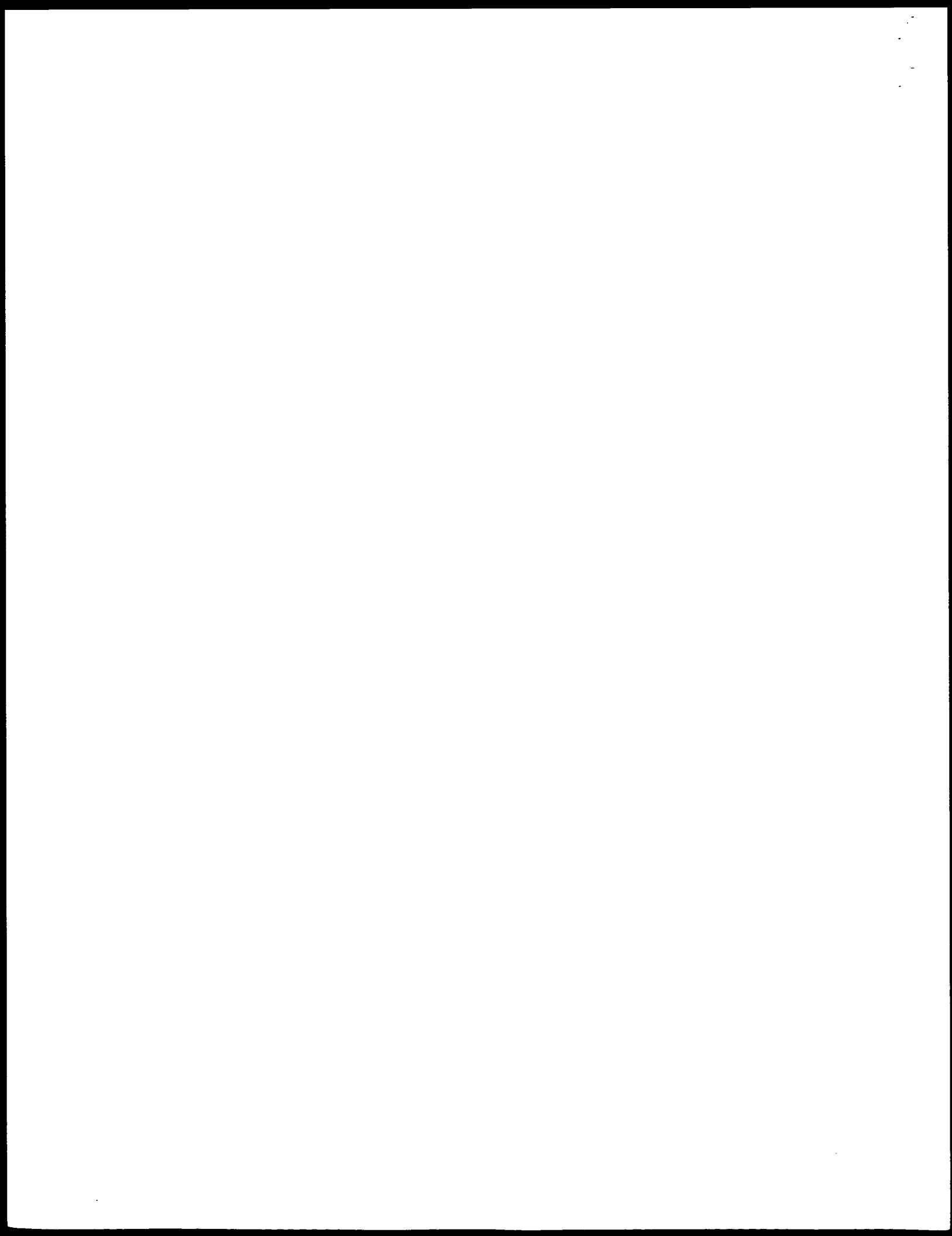
NH2-EQIAKYILYSHAVSSNGN-COHN$_2$.

61.   A drug delivery composition of claim 58,
wherein said amino acid sequence is

NH$_2$-IAKYILYSHAVSSNGNAVED-CONH$_2$.

62.   A drug delivery composition of claim 58,
wherein said amino acid sequence comprises amino acids
9-96 of E-cadherin.

63.   A drug delivery composition of claim 43,
wherein said conjugate comprises a physiologically-
cleavable covalent bond.

64.   A drug delivery composition of claim 43,
wherein said conjugate is encapsulated within a
physiologically-compatible particle.

65.   A drug delivery composition of claim 64,
wherein said particle comprises a liposome.

FIG. la.
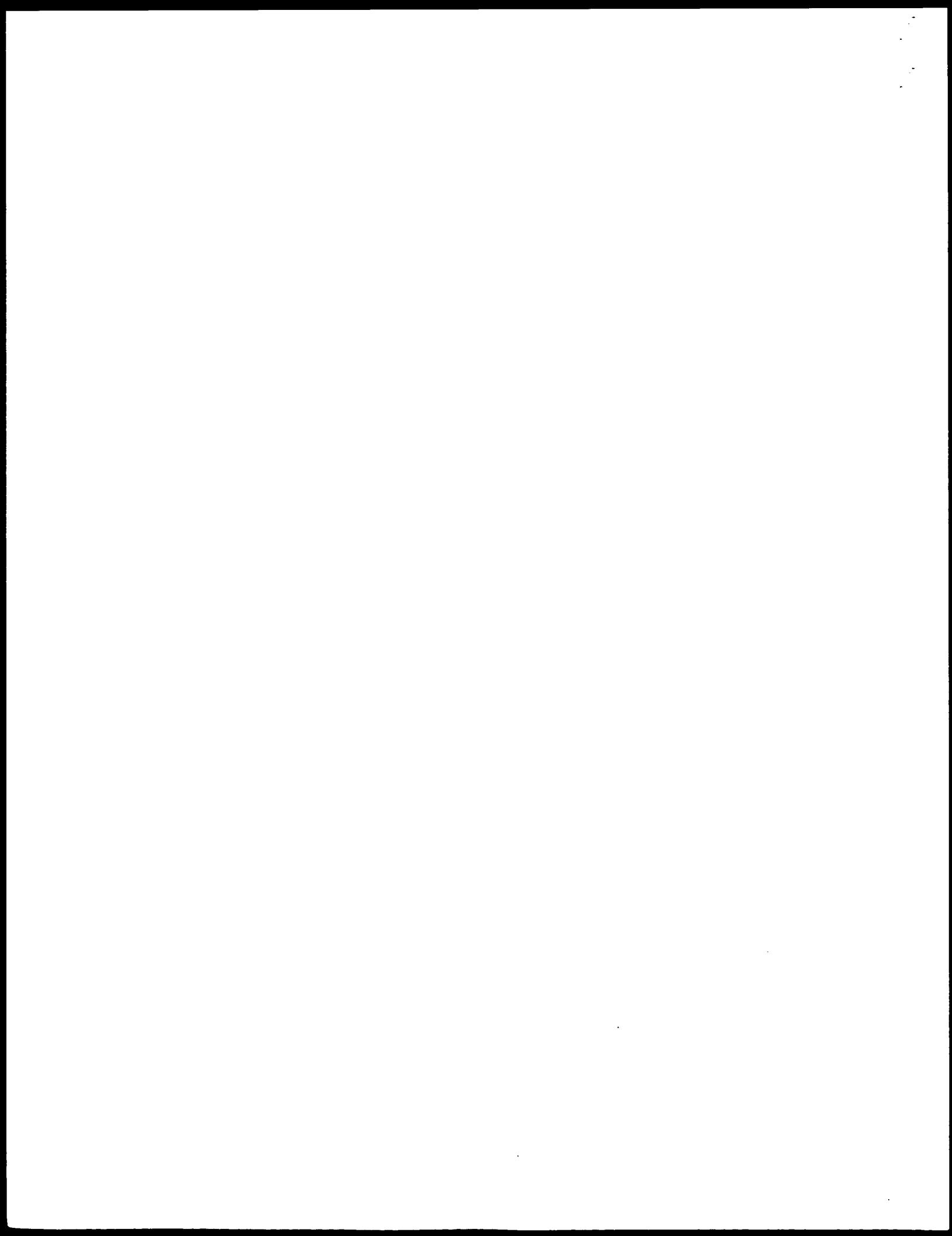
Partial cDNA sequence for the bovine endothelial N-cadherin

GAATTCGAAC CCCTTCGTTT CATTATGCAA GACTGGGATT CCTGAAGATG TGTACAGTGC   60

AGTCTTGTCC CGGGATGTGC TGGAAGGACA GCCCCTTCTC AATGTGAAGT TTAGCAACTG   120

CAATGGGAAA AGAAAAGTAC AGTATGAGAG CAGCGAGCCA GCAGATTTTA AGGTGGATGA   180

AGATGGCATG GTGTATGCCG TGAGAAGCTT CCCCCTCTCA TCTGAACACT CGAAGTTCCT   240

GATATACGCT CAAGACAAAG AGACTCAGGA GTAGCAGTAA AACTGAGCCT   300

CAAACCAGCC CTACCTGAGG ATTCAGTGAA GGAATCACGA GAAATAGAAG AAATAGTGTT   360

TCCAAGACAA GTGACTAAGC ACAATGGCTA CCTGCAGAGG CAGAAGAGAG ACTGGGTTAT   420

CCCTCCCATC AACTTGCCAG AGGGCCTTTT CCTCAAGAGC TCGTTCAGGAT   480

CAGATCCGAT AGAGATAAAA ACCTTTCTCT GCGGTACAGC GTAACTGGGC CAGGAGCTGA   540

CCAGCCTCCA ACTGGTATCT TCATTATCAA CCCCATCTCA GGTCAGCTGT CAGTAACCAA   600

GCCTCTGGAT CGTGAGCTGA TAGCCCGGTT TCATTTGAGG GCACATGCAG TGGATATTAA   660

TGGAAACCAA GTGGAGAACC CCATCGACAT TGTCATCAAC GTTATTGACA TGAATGATAA   720

CAGACCTGAG TTCTTACACC AGGTTTGGAA TGGGACAGTT CCTGAGGGAT CAAAGCCGGG   780

AACATATGTG ATGACGGTCA CTGCGATTGA TGCTGACGAT CCAAATGCCC TCAATGGGAT   840

FIG.1b.

```
GTTGAGGTAC AGAATCCTGT CCCAGGCGCC AAGCACCCCT TCGCCCAACA TGTTTACAAT   900
CAACAATGAG ACTGGGGACA TTATCACGGT GGCAGCTGGA CTTGACAGAG AAAAAGTACA   960
ACAGTATACG TTAATAATTC AAGCTACAGA CATGGAAGGC AATCCCACAT ATGGCCTTTC  1020
CAACACAGCC ACGGCTGTCA TCACGGTGAC GACAATCCTC            CGGAGTTTAC  1080
TGCCATGACG TTCTATGGTG AAGTCCCTGA GATGTCATCG            TCGCTAATCT  1140
AACAGTGACA GATAAGGATC AGCCCCACAC AACGCCATCT            ACAGAATCAG  1200
CGGTGGAGAC CCCGCCGGCC GCTTTGCCAT CCCAACAGCA            ACGACGGTTT  1260
AGTCACCGTA GTAAAACCAA TCGACTTTGA ATGTATGTCC            TTACTGTCGC  1320
TGCAGAAAAT CAAGTGCCAT TAGCCAAGGG CCACCTCAGT            CAACTGCGAC  1380
TGTGTCTGTC ACAGTTATCG ATGTGAATGA TTTGCCCCAA            ATCCAAAGAT  1440
CATTCGCCAA GAAGAAGGCC TTCACGCCGG TACCGTGTTA            CTGCTCAGGA  1500
CCCAGATCGA TATATGCAGC AAAATATCAG ATACACCAAA            CTGCAAACTG  1560
GCTAAAAATA GACTCTGTGA ATGGGCAGAT AACTACCATT GCTGTTTTGG ACAGAGAATC  1620
ACCGAATGTG AAAGCCAATA TATACAATGC TACTTTCCTT GCTTCTGACA ATGGAATCCC  1680
TCCTATGAGT GGAACGGGAA CACTGCAGAT CTATTTACTT GATATTAATG ACAATGCCCC  1740
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FIG. Ic.

```
TCAAGTGTTA CCTCAAGAGG CAGAGATTTG TGAAACTCCG GACCCCAATT CAATTAACAT 1800
CACAGCACTT GATTATGACA TTGATCCAAA TGCTGGACCA TTTGCTTTTG ATCTTCCTTT 1860
GTCTCCAGTG ACTATTAAGA GAAATTGGAC CATCACTCGG CTTAATGGTG ATTTTGCTCA 1920
GCTTAACTTA AAGATAAAAT TTCTTGAGGC CGGGATCTAC GAAGTTCCAA TCATAATCAC 1980
AGATTCGGGT AATCCTCCCA AATCGAATAT CTCCATCCTT CGGGTGAAGG TTTGCCAGTG 2040
TGATTCCAAC GGGGACTGCA CAGATGTGGA TCGAATTGTG GGAGCAGGGC TGGGCACCGG 2100
CGCCATCATC GCCATCCTGC TTTGCATCAT CATCCTGCTC ATTCTCGTTC TGATGTTCGT 2160
GGTATGGATG AAACGCCGGG ATAAAGAACG CCAGGCCAAA CAACTTTTAA TTGATCCAGA 2220
AGATGATGTA AGAGATAATA TTTTAAAATA TGATGAAGAA GGTGGAGGAG AAGAAGACCA 2280
GGACTACGAT TTGAGCCAGC TCCAGCAGCC TGATACGGTA GAGCCAGATG CCATCAAGCC 2340
AGTTGGAATC CGACGGTTGG ATGAGAGGCC CATCCATGCG GAGCCCCAGT ACCCGGTTCG 2400
ATCTGCAGCC CCACACCCAG GGGACATCGG GGACTTCATT AATGAGGGCC TTAAAGCTGC 2460
TGACAACGAT CCCACCGCTC CGCCCTACGA GTCTTTGACT ATGAAGGCAG               2520
TGGCTCCACG GCCGGGTCCT TGAGCTCCCT TAATTCCTCC AGTAGTGGAG GTGAGCAGGA 2580
CTATGACTAT CTGAACGACT GGGGGCCCCG CTTCAAGAAA CTCGCTGACA TGTACGGTGG 2640
```

FIG.ld

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AGGTGATGAC TGAACTTCAG GGTGAACTTG GTTTTGGGAC AAGTACAAAC AATTGCAACT 2700
GATATTCCCA AAAGCATTC  AGAAGCTAGG CTTTAACTTT GTAGTCTACT AGCACAGTGC 2760
TTGCTGGAGG CTTGGCCAGA GGCTGCAAAC CAATTGGGGC TCAGAGGGAA TATCGGTGAT 2820
CCAATACTGT TTGGAAAACA CTGAGCTCAG TTACACTTGA ATTTTACAGT ACAGAAGCAC 2880
TGGGATTTTA TGTGCCTTTT TGTACCTTTT TCAGATTGGA ATTAGTTTTA TGTTTAAGGC 2940
TTTAATGGTA CTGATTTCTG AAATGATAAG TAAAAGACAA AATATTTGT  GGTGGGAGCA 3000
GTAAGTTAAA CCATGATATG CTTCGACACG CTTTTGTTAC ATCGCATTG  CTTTTATTAA 3060
AAATATGGAA TTAAACAGAC AAACCAACCA CTCATGGAGC AATTTTATTA CCTTGGGGGC 3120
TGAGACCATG AGATTGGAAA ATGTACATTA TTTCTAGTTT TAGACTTTAG TTTCTTGTTT 3180
TGTTTTTTTT TTCCACTAAA ATCTTAAAAC TTACGCAGCT GGTTGCAAAT AAAGGGAGTT 3240
TTCATATCAC CAATTTGTAG CAAAATTGAA TTTTTTCATA AACTAGAATG TTAGACACAT 3300
TTTGGTCTTA ATCCATGTAC ACTTTTTTAT TTACTGTATT TTTTCCACTT CACTGTAAAA 3360
ATGGTATGTG TACATAATGT TTTATTGGCA TAGTCTATGG AGAAGTGCAG AAACTTCAGA 3420
ACATGTGTAT GTATTATTG  GACTATGGAT TCAGGTTTTT TGCATGTTTA TATCTTTCGT 3480
TATGGATAAA GTATTACAA  AACAAAGTGA CATTTGATTC AATTGTTGAG CTGTAGTTAG 3540
AATACTCAAT TTTTAATTTT TTTTAATTTT TTATTTTTA  TTTTCTCTTT TTGTTTGGGG 3600
```

```
AGGGAGAAAA GTTCTTAGCA CAAATGTTTT ACATAATTTG TACCAAAAAA AAACAAAAAA   3660
AAAGGAAAGA CAAGAAATGA AAGGGGTGAC CTGACACTGG TGGTACTACT GCAGTGTGTG   3720
TTTTTAAAAA AAAATGAAAA AAAAAAAGCT TTTAAACTGG AGAGACTTCT GACAACAGCT   3780
TTGCCCTCGT ATTGTGTACC AGAATATAAA TGATACACCT CTGACCCCAG CGTTCTGAAT   3840
AAAATGCTAA TTTTGGAAAA AAAAAAAAA AAAAA                              3875
```

FIG.1e.

FIG.2a.

partial cDNA sequence for the bovine endothelial P-cadherin

```
GAATTCGAAC CCCTTCGCTG AGAACACAGT GAGCCACGAG GTGCAGAGGC TGACAGTGAC    60
TGATCTGGAC GCCCCTAACT CACCAGCATG GCGTGCCACC TACCGCATCG TGGGAGGTGA   120
CAACGGGGAC CATTTTACCA TCACTACTGA CCCCGAGAGC AACCAGGGTA TCCTGACCAC   180
CCAGAAGGGC TTGGATTTTG AGGCCAAAAC CCAGCACACC CTGTACGTCG AAGTGATCAA   240
CGAGGTTCCC TTTGTGGTGA AACTCCCGAC ACCGTAGTGG TCCTCGTGGA              300
GGATGTGAAT GAGCCACCCG GTCATCGAAA TCCAGGAGGG                         360
CATCTCCACT GGGGAGCCTA TTTGTGCCTA CACTGCACGG GACCCAGACA AGGGGAGTCA   420
```

SUBSTITUTE SHEET

FIG.2b

```
GAAGATCAGT TACCACATCC TGAGAGACCC AGCAGGGTGG CTAGGCGATGG ACCCAGACAG   480
TGGACAAGTC ACTGCCGCAG GGGTCTTGGA CCGTGAGGAT GAGCAGTTTG  TGAGAAACAA   540
CATCTACGAA GTCATGGTCT TGGCCACAGA TGATGGGAGC CCTCCCACCA  CTGGCACAGG   600
GACCCTCCTG CTAACACTGA TGGACATCAA TGACCACGGT CCGGTCCCCG  AGCCCCGTCA   660
GATCACCATC TGCAACCAAA GCCCTGTGCC CCAGGTGCTA AACATCACAG  ACAAGGACTT   720
GTCCCCCCAC ACTGCCCCTT TCCAGGCCCA ACTCACACAT GACTCGGACG  TCTATTGGAC   780
AGCAGAAGTC AACGAGAAAG GAGACGCAGT AGCCTTGTCC CTGAAGAAGT  TCCTAAAGCA   840
AGGCGAATAC GATGTGCACC TTTCCCTGTC AACAAGGAAC AGCTGACAGT  ACCCCTGGAC   900
GATCAGAGCC ACCGTGTGTG ACTGCCACGG CAACATGGTG ACCTGCCGGG  ACCCCTGGAC   960
GTGGGGTTTC CTCCTCCCCA TCCTGGGTGC TGCCCTGGCT CTGCTGCTCC  TTCTGCTGGT  1020
GCTCCTATTC TTGGTGAGAA AGAAACGGAA GATCAAGGAA CCCCTTCTCC  TCCCAGAAGA  1080
TGATACCCGT GACAAGTCT  TCTACTACGG CGAAGAGGGG GGTGGCGAGG  AGGACCAGGA  1140
CTATGACATC ACCCAGCTCC ACCGGGTCT  GGAGGCCCGG CCTGAGGTGG  TTCTCCGCAA  1200
CGATGTGGCA CCATCCTTCA TCCCCACACC CATGTACCGT CCTCGGCCAG  CCAACCCAGA  1260
TGAAATCGGC AACTTCATCA TTGAGAACCT GAAGGCAGCC AACACAGACC  CCACGGCCCC  1320
```

SUBSTITUTE SHEET

```
GCCCTACGAC TCCCTGTTGG TGTTCGACTA TGAGGGCAGT GGCTCCGATG CCGCCTCTCT  1380
GAGCTCGCTC ACCTCCTCAA CCTCTGACCA GGACCAAGAC TACAACTATC TGAATGAGTG  1440
GGGCAGCCGC TTCAAGAAGC TGGCGGACAT GTACGGGCGG GGCCAGGACG ACTAGGACTC  1500
CCTAAACGCC GGGCTGCAGC AGGGTCTCCA AGCGGTCACT ATCCCCACGT TGGCCAAGGA  1560
CTTTGCAGCT TGTTGAGAAT TGGCCTTAGC AACTTGGAGG GAAGAGGCCT CGAAACTGAC  1620
CTCAAAGGGG CAGGTCTCTA TGCCTTTCAG AACGGAGGAA CGTGGGCAGT TTGATTTCAA  1680
CAGTGAGCAC CTCTTAGCCT AAGCCAGGGC TGCTCAATTT CTGGGAGTCT CCTCGCTACC  1740
ATAAAATGCT CAGCGCTGGG TCCTGGGTTT TGACTGACTC TGACTTTCCC ATGATGGCTT  1800
TTGCTCTGGA ATGGACCCTT CTCCTTAGTA ACAGGCCTCT TACCACAATC TTCGTTTTTT  1860
TTTTTTTAAT GCTGTTTTCA AAAAGTGAGA GGCAGGTCCT CAACCACCCC CTGGAGCGCT  1920
CCAGAAGCCC AGGCGTGCCC TCTCTGTGGTC CTCTGTGGTC TCTTGGCCCC CAGACCTCCT  1980
GTTTGATTGG ATAACTGCAT TTTTATACTG AGCACGTCTA AGTGGTCCTT TATTTTTTAT  2040
TTTCCCTATC GAGTGCTGTA GATGAAGAGT GATGACAATC CTGTAAATGT ACTAGAACTT  2100
TTTTATTAAA CCCAAAAAA GGAACTTTTT AAAAAAAAA AAAAAAAAA AAAAAC        2156
```

FIG. 2c.

FIG. 3a.

cDNA sequence for MDCK E-cadherin

```
CGGGCACCTG TGATTCGGCGG AAGTCCTGCC GCCTCGGCGCC GCCTCGGCGCC CGGCTCTCGA   60
CCCCGCCCCG CCATGGGCCC  TCGGTACGGC GGGGCTCTGC  CGCTCCTGCT  CCGCTGCTG   120
CTGCTGCTGC AGGTCTCATC  GGGGCTCTGC AGCCCTGCCG  AGCCCTGCCG  CCTGGCTTT   180
GGGCCTGACA GCTACACGTT  CACCGTGCCC CGGCGACACT  TGGAGAGAGG  CCGTGTCCTG   240
GGCAGGGTGA GTTTGAAGG   ATGCACCGGT CTACCTAGGA  CAGCCTATGT  TTCTGATGAC   300
ACCCGATTCA AAGTGGGCAC  AGATGGTGTG ATTACAGTCA  AGCGGCCTCT  ACAACTTCAT   360
AAACCAGAGA TAAGTTTTCT  TGTCCATGCC TGGGACTCCA  GCCGCAGGAA  GCTCTCCACC   420
AGAGTTAGGC TGAAGGCAGC  GACGCACCAC CACCACCACC  ATCATGATGC  TCCCTCTAAA   480
ACCCAGACAG AGGTGCTCAC  ATTCCCAGT  TCCCAGCATG  GACTCAGAAG  ACAGAAGAGA   540
GACTGGGTTA TCCCTCCTAT  CAGCTGCCCG GAAAACGAGA  AAGGCCCATT  TCCTAAAAAC   600
CTGGTTCAGA TCAAGTCTAA  CAGGGACAAA GAAATCAAGG  TTTTCTACAG  CATCACTGGC   660
CAAGGAGCTG ACGCACCTCC  TGTTGGTGTG TTTATTATTG  AAAGAGAAAC  AGGATGGCTG   720
AAGGTGACTG AGCCTCTGGA  TAGAGAACAA ATTGCTAAGT  ACATTCTCTA  CTCTCATGCC   780
GTATCTTCTA ATGGGAATGC  GGTTGAAGAC CCAATGGAGA  TCGTGATCAC  GGTGACAGAT   840
```

FIG. 3b.

```
CAGAATGACA ACAAGCCCGA GTTCACCCAG GCAGTCTTCC AAGGATCTGT CACGGAAGGT   900
GCCCTTCCAG GCACCTCTGT GATGCAGGTG ACAGCCACAG ATGCGGGATGA TGATGTGAAT   960
ACCTACAACG CTGCCATCGC TTACAGCATC CTCACACAAG ACCCCCTCCT GCCTAGCAGC  1020
ATGATGTTCA CTATCAACAA GGACACAGGA GTCATCAGCG TGCTCACCAC TGGGCTGGAC  1080
CGAGAGGGTG TCCCCATGTA CACCTTGGTG GTTCAGGCTG CTGACCTGCA AGGCGAAGGC  1140
TTAACTACAA CTGCAACAGC TGTGATCACA GTCACTGACA TCAATGATAA CCCCCCCATC  1200
TTCAACCCAA CCACGTACCA GGGACGGGTG CCTGAGAACA AGGCTAACGT CGAAATCGCT  1260
GTACTCAAAG TGACGGATGC TGATGTCCCC GATACCCCGG CCTGGAGGGC TGTGTACACC  1320
ATATTGAACA ATAACAATGA TCAATTTGTT GTCACCACAG ACCCAGTAAC TAACGACGGC  1380
ATTTGAAAAA CAACTAAGGG CTTGGATTTT GAGGACAAGC AGCAGTATGT CTTGTACGTG  1440
ACTGTGGTGA ACGTGACCCC GTTTGAGGTC ATCCTCTCCA CCTCCACAGC CACTGTCACT  1500
GTGGACGTGG AAGATGTGAA TGAAGCCCCC ATCTTCATCC CTTGCCCAAA GGTAGTGTCA  1560
ATCCCTGAAG ACTTTGGTGT GGGCCAGGAA ATCACATCCT ACACCGCCGA GGATCCAGAT  1620
ACATATATGG AACAGAGGAT AACGTATCGG ATTTGGAGGG ATGCTGCCGG TTGGCTGGAG  1680
GTTAATCCAG AATCTGGTGC CATTTTCACT CGGGCTGAGC TGGACAGAGA GGATTTTGAG  1740
```

FIG. 3c.

```
CACGTGAAGA ATAGCACGTA TGAAGCCCTC ATTATAGCCA TTGACTTCGG TTCTCCAGTT 1800
GCTACTGGAA CGGGAACTCT TCTACTGGTC CTCTCTGATG TGAATGACAA TGGCCCCATT 1860
CCAGAACCTC GAAATATGGA CTTCTGCCAG AAAAACCCAC AGCCTCATGT CATCAACATC 1920
ATTGATCCAG ATCTTCCCCC CAACACATCT CCCTTCACAG CAGAACTAAC ACACGGCGCA 1980
AGTGTCAACT GGACCATCGA GTACAATGAC CCAGCTCGTG AATCTCTAAT TTTGAAGCCA 2040
AAGAAAACTT TAGAGTTGGG TGACTACAAA ATAAATCTCA AGCTCACAGA TAACCAGAAC 2100
AAGGACCAGG TGACCACCCT ATATGTGTTT GTGTGCGACT GCGAAGGTGT CGTCAACAGC 2160
TGCAAGAGGA CGGCGCCTTA CGCCGAAGCA GGCTTGCAGG TTCCTGCCAT CTTGGGCATT 2220
CTCGGAGGAA TCCTCGCTCT ATTCTGCTGC TTCTGCTATT TGTTCGGAGG 2280
AGAAGGGTGG TCAAAGAGCC CTTACTTCCC CCAGAAGATG ACACCCGGGA CAATGTTTAT 2340
TACTATGATG AAGAAGGAGG TGGAGAGGAG GATCAGGACT TTGACTTGAG CCAGTTGCAC 2400
AGGGGCCTGG ATGCTCGGCC TGAAGTGACT CGCAATGATG TGGCCCCAAC CCTCCTGAGT 2460
GTGCCCCAGT ATCGGCCCCG CCCTGCCAAT CCTGATGAAA TTGGAAACTT TATTGATGAA 2520
AACCTGAAGG CAGCGGACAC TGACCCTACT GCTCCTCCTT ATGACTCTCT GCTCGTGTTT 2580
GACTATGAAG GAAGCGGGTC TGAAGCTGCT AGTCTGGAGCT CCTTGAACTC CTCAGAGTCA 2640
GACCAAGACC AGGACTATGA CTACCTGAAT GAATGGGGCA ATCGCTTCAA GAAGCTGGCG 2700
```

FIG. 3d.

```
GACATGTATG GAGGTGGCGA GGACGACTAG GGGACTTGAG ACAAATGAAG ATGAGTCCTT  2760
ATACCATGTG GTAGAAAATG CGGAGGTGAC TGTTTTCAGC TCCCTTCATC TGAGAGGAAT  2820
TTCTGGAGAA GAGAAAATGC ACAGTGATAT ATAGTTAGGA TAGTTAGGAT TTCTACTTTA  2880
TAGATCTAAT CTGTGTGTT  GTTAGAACGA TTTTGACCTA TTCTTTGAAG CTTTTTTTTC  2940
TTTCTTTCAT CATTCTTTAA ATGGTGATGC TGTCCAAAAG ACCCCCCACA TGTTTATATT  3000
TCAAAGAAT  AGCTAAAGCC TCCAGAAGGT TCTGCTAGCA ATTTCGAGAT TGCCTTATTG  3060
ACTTGTCTCA TTTTTTTAAA GGAAGGTAGG GCTAAACTAC CCTATTGTGT TTGTGTGTGT  3120
GTGTGTGTAT GTGTAATTAT TTTTAATTTG TGTTCTTTTT TCTCCTATCA CTGCACTGGT  3180
GTCCCGTGTT CTAATAACCA CTCTTAACTC CTTCTGAACT TACATTGCCT CAGACAGGAG  3240
TTCTCTGCTG CAGAAATTAT TGGGCCCTT  CAGGATAAGA GACTTGGTCT TAGTTTGATG  3300
GTAGTGTGAC TGGGTATTAT GGACTCGTAA GGACTTTAGT GGTTCTCCTT TTTTATTTCC  3360
TAAGTACATA AATTGAAATT CATATCCATC CACTGACTTG TTCTGCATTA AGTGTGTTTG  3420
TCATGTGGAC GTCATTATTG GGCTACTTTG GTTCTGAACA AGGAGCATTG ACCAGAAAAG  3480
GTGGTGAATT TTCAGGTGCC ACTCAACTTC TAATGTTCAC TTATCACTCA AACAGAGAG   3540
TGATCTATTC TGACGTTTAG CGTAGTGCCT GCAGTGCTGC AGCCAAAGAT TGAAGGCGGA  3600
```

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```
TTGTCAAAGC CAAGGGCAAC ATGAAAAATG GACTTGGGAGG TGGCAGGCGG GATGGGTCAT  3660
TGAGCCTGGC GTTTTAGCAA ACTGATGCTG AGGATAACTG AGGTGGCTCT ACCTCTAGTC  3720
CTGAAAATTC TGAAGAATGG AAGAATCCCG ACAAGTGTGT CCTTATCGCGA TCCTTAGGTC  3780
ACAGTTTGTA CCTGAGGCCA AGAATCCCCA GGTGCCTGCT TTTGTTAATG TCTACCGAAA  3840
ATGCAGCCTG ATCTGGACTC AGGTGCCCCA ATTCTAAGTG TGCATAGAAA ACTGACAATA  3900
TTAGGAAATT CTTTTTCCCC CCTTAGGAGC AGGAAGAAAA TATGACCCTA AAGGGTTTG  3960
GCAAAGGGAA GGTGGGGAGA GCTTTGACTT GGATTTTTT TAAATTGAAA TGTGAACTTC  4020
AAGGAACTTT TGACAACCAT GGGAAATAAT TTTATCTTAA ATTGCTTTAC TGTCTGTCAG  4080
CTGTTTTTCA AAGAAAAAA AAATCATCCC TGCAATCACT TCTTGGAATT GTCTTGATTT  4140
TTCAGCAATT TAAACTCTAA TTTAGTCCTG TATAGAGAAT GTTAATGTAG TTTTGAGTGT  4200
ATATGTGTGT GGGTACGGAT AATTTTGTAT TTTCTTTAGG TCTGGAAAAG GAAAACAATT  4260
TAAGCTGCGA AAATTCTTAA ATATTCATTT TTATAAATTT TATTAAAGAA TTTTGTTAAA  4320
AAAAAAAA AAA                                                        4333
```

FIG.3e.

WO 91/04745

FIG. 4a.

N-cadherin restriction map

```
EcoRI
 AsuII
  BstBI   XmnI
 |        |
GAATTCGAACCCCTTCGTTTCATTATGCAAGACTGGATTCCTGAAGATGTGTACAGTGC    60

      SmaI
       XmaI
       AvaI
       |
AGTCTTGTCCGGGATGTGCTGGAAGGACAGCCCCTTCTCAATGTGAAGTTTAGCAACTG   120

CAATGGGAAAGAAAAGTACAGTATGAGAGCCAGCCAGCAGATTTAAGGTGGATGA       180

            HindIII
            |
AGATGGCCATGGTGTATGCCGTGAGAAGCTTCCCCCTCTCATCTGAACACTCGAAGTTCCT  240

       SauI
       Eco81I
       Bsu36I
       EcoNI
       |
GATATACGCCTCAAGACAAAGAGACTCAGGAAAGTGGCAAGTAGCAGTAAAACTGAGCCT  300

CAAACCAGCCCTACCTGAGGATTCAGTGAAGGAATCACGAGAAATAGAAGAAATAGTGTT  360
```

FIG.4b.

```
                                                                        420
TCCAAGACAAGTGACTAAGCACACAATGGCTACCTGCAGAGGCCAGAAGAGAGAGACTGGGTTAT
                                  |      |
                                 PstI   BspMI

                                                                        480
CCCTCCCCATCAACTTGCCAGAAAACTCCAGAGGGCCTTTCCTCAAGAGCTCGTCAGGAT
                   |                                 |       |
                 EcoO109                            SstI    AlwNI
                 EaeI                               SacI
                 DraII                              HgiAI
                                                    Bsp1286
                                                    BanII

                                                                        540
CAGATCCGATAGAGAGATAAAAACCTTTCTCTGCGGTACAGCGTAACTGGGCCAGGAGCTGA
   |                                              |
 XhoII                                          PvuII

                                                                        600
CCAGCCTCCAACTGGTATCTTCATTATCAACCCCATCTCCAGGTCAGTGTCAGTAACCAA
   |                                       |         |
 BstXI                                   Bsp1286    AseI
                                         NspHI
```

FIG.4c.

```
GCCCTCTGGATCGTGAGCTGATAGCCCGGTTCATTTGAGGGCACATGCAGTGGATATTAA   660
                          |
                        Tth111I

TGGAAACCAAGTGGAGAACCCCATCGACATTGTCATCAACGTTATTGACATGAATGATAA   720
                                                  SauI
                                                  Eco81I
                                                  Bsu36I
                                                  AlwNI
                                                  ||

CAGACCTGAGTTCTTACACCAGGTTTGGAATGGGACAGTTCCTGAGGGATCAAAGCCCGGG   780
     |
   NdeI

AACATATGTGATGACGGTCACTGCGATTGATGCTGACGATCCAAATGCCCTCAATGGGAT   840
                          HaeII
                          BbeI
                      NarI
                      BanI  AhaII
                  EcoNI     ||
                    |

GTTGAGGTACAGAATCCTGTCCCAGGCGCCAAGCACCCCTTCGCCCAACATGTTTACAAT   900
                                              NspHI
                                            AflIII
                                              |
                      PvuII
                        |

CAACAATGAGACTGGGGACATTATCACGGTGGCAGCTTGACAGAGAAAAGTACA          960
```

FIG. 4d.

```
                  AccI                                NdeI
ACAGTATACGTTAATAATTCAAGCTACAGACACATGGAAGGCAATCCCACATATGGCCTTTC  1020

                       HincII               BspMII
                                            AccIII
CAACACAGCCACGGCTGTCATCACGGTGACAGATGTCAACGACAATCCTCCGGAGTTTAC  1080

TGCCATGACGTTCTATGGTGAAGTCCCTGAAAAACAGGGTAGATGTCATCGTGCTAATCT  1140

                           Cfr10I
AACAGTGACAGATAAGGATCAGCCCCACACCGGCCTGGAACGCCATCTACAGAATCAG  1200

       NaeI
       Eco52I
       EagI
       Cfr10I
CGGTGGAGACCCGCCGGCCGCTTTGCCATTCAAACTGACCCCAACAGCAACGACGGTT  1260

                           StyI
AGTCACCGTAGTAAACCAATCGACTTTGAAACAAATAGGATGTATGTCCTTACTGTCGC  1320

       PstI                    HincII
TGCAGAAAATCAAGTGCCATTAGCCAAGGGTATTCAGCATTCCACCTCAGTCAACTGCGAC  1380
```

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FIG. 4e.

```
                                  ClaI
Tth111I                            |
|                                  
TGTGTCTGTCACAGTTATCGATGTGTGAATGAAAATCCTTATTTGCCCCAAATCCAAAGAT   1440

                                                        EcoO109
                    BanI                                 DraII
              XmnI  Asp718   HpaI                          |
              StuI  Cfr10I KpnI HincII
         EaeI  |      |     |    |
          |    |      |     |    |
CATTCGCCAAGAAGAAGGCCCTTCACGCCCGGTACCGTGTTAACAACGTTTACTGCTCAGGA   1500

     ClaI
      |
CCCAGATCGATATGCAGCAAAATATCAGATACACCAAATTATCCGATCCTGCAAACTG      1560

GCTAAAAATAGACTCTGTGAATGGGCAGATAACTACCATTGCTGTTTGGACAGAGAATC     1620

ACCGAATGTGAAAGCCAATATATACAATGCTACTTTCCTTGCTTCTGACAATGGAATCCC    1680

         XhoII
         PstI
         BglII
          |                                          AseI
          |                                           |
TCCTATGAGTGGAACGGGAACACTGCGAGATCTATTTACTTGATATTAATGACAATGCCCC   1740

                                                    BspMII
                                                    AccIII
                                                      |
TCAAGTGTTACCTCAAGAGGCAGAGATTGTGAAACTCCGGACCCCCAATTCAATTAACAT    1800
```

FIG. 4f.

```
PflMI
                  |
CACAGCACTTGATTATGACACATTGATCCAAATGCTGGACCATTTGCTTTTGATCTTCCTTT   1860

                                                        CelII
                                                           |
GTCTCCAGTGACTATTAAGAGAAATTGGACCATCACTCGGCTTAATGGTGATTTGCTCA   1920

              XhoII
                 |
GCTTAACTTAAAGATAAAATTTCTTGAGGCCCGGGATCTACGAAGTTCCAATCATAATCAC   1980

AGATTCGGGTAATCCTCCCAAATCGAATATCTCCATCCTTCGGGTGAAGGTTGCCAGTG   2040

                                               Cfr10I
                                               Bsp1286
                                                BanI  BanI
                                                  |     |
TGATTCCAACGGGGACTGCACAGATGTGGATCGAATTGTGGGAGCAGGGCTGGGCACCGG   2100

HaeII
BbeI
NarI
AhaII
 |
```

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FIG.4g.

CGCCATCATCGGCCATCCTGCTTTGCATCATCATCCTGCTCATTCTCGTTCTGATGTTCGT   2160

GGTATGGGATGAAACGCCGGGGATAAAGAAACGCCAGGCCAAACAACTTTAATTGATCCAGA   2220

            DraI
        SspI AhaIII

AGATGATGTAAGAGATAATATTTTAAAATATGATGAAGAAGTGGAGGAGAAGAAGACCA   2280

GGACTACGATTTGAGCCAGCTCCAGCAGCCTGATACGGTTAGAGCCAGATGCCATCAAGCC   2340

                              Bsp1286
                              BanII
       EaeI

AGTTGGAATCCGACGGTTGGATGAGAGGCCCATCCATGCGGAGCCCCAGTACCCGGTTCG   2400

                       EcoO109
                       EaeI
                AseI   DraII

ATCTGCAGCCCCACACCCAGGGGACATCGGGACTTCATTAATGAGGCCCTTAAAGCTGC   2460

PstI

TGACAACGATCCCACCGCTCCGCCCTACGACTCCCTCTTAGTCTTTGACTATGAAGGCAG   2520

                   SstI
                   SacI
               HgiAI
     EcoO109 Bsp1286
 Eco52I   BanII
 EagI DraII

TGGCTCCACGGGCCGGGTCCTTGAGCTCCCTAATTCCTCCAGTAGTGGAGGTGAGCAGGA   2580

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FIG. 4h.

```
                        Bsp1286
                        BanII
                        ApaI
                        EcoO109
                        DraII
                        EcoO109
                        EaeI
                        DraII              AflIII  NspHI
CTATGACTATCTGAACGACTGGGGGCCCCGGCTTCAAGAAACTCGCTGACATGTACGGTGG  2640

AGGTGATGACTGAACTTCAGGGTGAACTTGGTTTTTGGACAAGTACAAACAATTGCAACT  2700
                    BsmI                          AccI
GATATTCCCAAAAAGCATTCAGAAGCTAGGCTTTAACTTTGTAGTCTACTAGCACAGTGC  2760
                                                Bsp1286
                                                BanII
                      AlwNI                     SstI
                                                SacI
                                                HgiAI
                                                Bsp1286
TTGCTGGAGGCTTTGGGCAGAGGCTGCAAACCAATTTGGGCTCAGAGGGAATATCGGTGAT  2820
```

FIG.4i.


```
                                                          BanII
                                                          |
CCAATACTGTGTTGGAAAACACTGAGCTCAGTTACACTTGAATTTACAGTACAGAAGCAC        2880

TGGGATTTTATGTGCCTTTTTGTACCTTTTCAGATTGGAATTAGTTTATGTTTAAGGC         2940

                                  SspI
                                  |
TTTAATGGTACTGATTCTGAAATGATAAGTAAAAGACAAATATTTGTGGTGGGAGCA          3000

GTAAGTTAAACCATGATATGCTTCGACACGCTTTGTTACATCGCATTGCTTTTATTAA         3060

                                              StyI
                                              |
AAATATGGAATTAAACAGACAAACCAACCACTCATGGAGCAATTTTATTACCTTGGGGGC       3120

          BstXI
          |
TGAGACCATGAGATTGGAAAATGTACATTATTTCTAGTTTAGACTTTAGTTTCTTGTTT        3180

                                       PvuII
                                       |
TGTTTTTTTTCCACTAAAATCTTAAAACTTACGCAGCTGGTTGCAAATAAAGGGAGTT         3240

                       XmnI
                       |
TTCATATCACCAATTTGTAGCAAAATTGAATTTTTCATAAACTAGAATGTTAGACACAT        3300

TTTGGTCTTAATCCATGTACACTTTTTATTTACTGTATTTTTTCCACTTCACTGTAAAA        3360

ATGGTATGTGTACATAAATGTTTTATTGGCATAGTCTATGGAGAAGTGCAGAAACTTCAGA      3420
```

FIG.4j.

```
NspHI
AflIII
ACATGTGTATGGTATTATTGGACTATGGATTCAGGTTTTTGCATGTTTATATCTTTCGT   3480

TATGGATAAAGTATTACAAAACAAAGTGACATTTGATTCAATTGTTGAGCTGTAGTTAG   3540

AATACTCAATTTTTAATTTTTTATTTTCTCTTTTGTTTGGGG                    3600

AGGGAGAAAGTTCTTAGCACAAATGTTTACATAATTTGTACCAAAAAAAACAAAAAA     3660

                              BstEII            PstI
AAAGGAAAGACAAGAAATGAAAGGGGTGACCTGACACTGGTGGTACTACTGCAGTGTG    3720

DraI                          DraI
AhaIII                        AhaIII
                              HindIII
TTTTTAAAAAAAAATGAAAAAAAAAGCTTTAAACTGGAGAGACTTCTGACAACAGCT     3780

TTGCCTCTGTATTGTGTACCAGAATATAAATGATACACCCTCTGACCCCAGCGTTCTGAAT  3840

AAAATGCTAATTTTGGAAAAAAAAAAAA                                  3875
```

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FIG.4k.

P-cadherin restriction map

```
        BstBI                              DraIII  AlwNI  AlwNI
EcoRI   AsuII   XmnI
GAATTCGAACCCCTTCGCTGAGAACACAGTGTGAGCCACCAGAGGCTGCAGAGGCTGACAGAGTGAC   60

        AhaII
TGATCTGGACGCCCCTAACTCACCAGCATGGCGGTGCCACCTACCGCATCGTGGGAGGTGA         120

                            AvaI
CAACGGGACCATTTACCATCACTACTGACCCCGAGAGCAACCAGGTATCCTGACCAC            180

                                        BclI
CCAGAAGGGCTTGGATTTTGAGGCCAAAACCAGCACACCCTGTACGTCGAAGTGATCAA          240

                            BstXI    EcoNI
CGAGGTTCCCTTTGTGGTGAAACTCCCGACCTCCAGCCACCGTAGTGGTCCTCGTGGA           300

                    EcoO109
                    DraII
GGATGTGAATGAGCCACCGTGTTGTCCCCCCGTCCAAAGTCATCGAAATCCAGGAGG            360

CATCTCCACTGGGGAGCCTATTTGTGCCTACACTGCACGGGACCCAGACAAGGGGGAGTCA         420
```

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FIG. 41.

```
                                    NheI
                          BstXI                      PflMI
GAAGATCAGTTACCACATCCTGAGAGACCCAGCAGGGTGGCTAGCGGATGGACCAGACAG  480

TGGACAAGTCACTGCCGCAGGGGTCTTGGACCGTGAGGATGAGCAGTTTGTGAGAAACAA  540

                 BalI              Bsp1286
                                   BanII
CATCTACGAAGTCATGGTCTTGGCCACAGATGATGGGAGCCCTCCCACCACTGGCCACAGG  600

Eco0109                                        Bsp1286
DraII                               AvaI       BanII
GACCCTCCCTGCTAACACTGATGGACATCAATGACCACGGTCCGGTCCCCGAGCCCCGTCA  660

                Bsp1286                             AatII
                                                    AhaII
GATCACCATCTGCAACCAAAGCCCTGTGCCCCAGGTGCTAAACATCACAGACAAGGACTT  720

        EaeI
GTCCCCCCACACTGCCCCTTCCAGGCCCAACTCACACATGACTGGACGTCTATTGGAC  780

HincII                          XmnI
AGCAGAAGTCAACGAGAAAGGAGACGCAGTAGCCTTGTCCCTGAAGAAGTTCCTAAAGCA  840
```

FIG. 4m.

```
                    HgiAI
                    Bsp1286
         ApaLl                                        PvuII
         |                                            |
AGGCGAATACGATGTGCACCCTTCCCTGTCCGACCACGGCAACAAGGAACAGCTGACAGT    900

                                                     BspMI
                                                     EcoO109
      DraIII            BstEII                       DraII
Bcl I  |                |                             | |
|     GATCAGAGCCACCGTGTGACTGCCACGGCAACACATGGTGACCTGCCGGGACCCCCTGGAC    960

GTGGGGTTCCCTCCCTCCCCATCCCTGGGTGCTCCTGGCTCTCGCCCTGCTCCTTCTGCTGGT    1020

            XmnI
HgiAI       |
Bsp1286     |
|          |
GCTCCTATTCTTGGTGAGAAAGAAGATCAAGGAACCCCTTCTCCTCCCAGAAGA    1080

Tth111I
|
|
TGATACCCGTGACAACGTCTTCTACTACGGGCAAGAGAGGGGGTGGCGAGGAGGACCAGGA    1140

                    SauI
                    Eco81I
            EaeI    Bsu36I
            |       |
CTATGACATCACCCCAGCTCCACCGGGGGTCTGGAGCCCGGCCTGAGGTGGTTCTCCGCAA    1200
```

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FIG. 4n.

BanI

CGATGTGGCACCATCCTTCATCCCCACACCCATGTACCGTCCTCGGCCAGCCAACCCAGA 1260

TGAAATCGGCAACTTCATCATTGAGAACCTGAAGGCAGCCAACACAGACCCCACGGCCCC 1320

GCCCTACGACTCCCCTGTTGGTGTTCGACTATGAGGGCAGTGGCTCCGATGCCGCCTCTCT 1380

SstI
SacI
HgiAI
Bspl286
BanII

GAGCTCGCTCACCTCCTCAACCTCTGACCAGGACCAAGACTACAACTATCTGAATGAGTG 1440

NspHI
AflIII

GGGCAGCCGCTTCAAGAAGCTGGCGGACATGTACGGGCGGCCAGGACGACTAGGACTC 1500

PstI   StyI

CCTAAACGCCGGGCTGCAGCGTCTCCAAGGGGTCACTATCCCCACGTTGGCCAAGGA 1560

StyI
BalI

StuI
EaeI

CTTTGCAGCTTGTTGAGAATTGGCCTTAGCAACTTGGAGGGAAGAGGCCTCGAAACTGAC 1620

FIG.40.

BspMI
|
CTCAAAGGGGCAGGTCTCTATGCCTTTCAGAACGGAGGAACGTGGGCAGTTTGATTTCAA　1680

HgiAI
Bsp1286　　EcoNI
|　　　　　　|
CAGTGAGCACCTCTTAGCCTAAGCCAGGGCTGCTCAATTTCTGGGAGTCTCCTCGCTACC　1740

EcoO109
DraII
Eco47III
CelII HaeII
|　　|
ATAAAATGCTCAGCGGCTCCTGGGTCCTGGGTTTGACTGACTCTGACTTTCCCATGATGGCTT　1800

StuI
EaeI
|
TTGCTCTCTGGAATGGACCCTTCTCCTTAGTAACAGGCCCTCCTTACCACAATCTTCGTTTTT　1860

HaeII
EcoO109　Pf1MI Eco47III
DraII　　　　|
BspMI　|　　　|
|　　|
TTTTTTAATGCTGTTTCAAAAAGTGAGAGGCAGTCCTCAACCACCCCCTGGAGCGCT　1920

Bsp1286 NsiI
|
CCAGAAGCCCCAGGCGTGCCCTCATGCCATTCTTCTGTGGTCTCTTGGCCCCCAGACCTCCT　1980

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FIG. 4p.

```
                                              HgiAI
                                              Bsp1286
                                                 |
GTTTGATTGGATAACTGCATTTTTATACTGAGCACGTCTTAAGTGGTCCTTTATTTTTAT   2040

TTTCCCTATCGAGTGCTGTAGATGATGAAGAGTGATGACAATCCTGTAAATGTACTAGAACTT   2100

        XmnI
         |
TTTTATTAAAGGAACTTTTCCCAAAAAAAAAAAAAAAAAAAAAAAAAAAAC   2156


   E-cadherin restriction map

BanI
 |                                          HaeII
 |                                          BbeI
 |                                 NarI
 |                                 AhaII
 |                                 BanI
 |                                   |
CGGGCACCTGTGATTCGCGGAAGTCCTGCCGCCTCGGCGCCCGGCTCTCGA   60

              BanII
              ApaI
         EaeI  |                                   CCCCCGCCCGGCCCATGGGCCTCGGTACGGGCGCCCCCGGCTCCCGGCTG   120
     StyI EcoO109
     NcoI DraII
        |  |
                 PstI
BspMI    |       BanII
  |      |         |
CTGCTGCTGCAGGTCTCATCGGGGCTCTGCCAAGAGCCGGAGCCCTGCCGCCCCTGGCTTT   180
                    BanII
                    BglI
```

FIG.4q.

```
                HaeII   AflIII
                 —        —
GGCGCTGACAGCTACACGTTCACCGTGCCCCGGGCGACACTTGGAGAGAGGCCGTGTCCTG  240

                        StyI
                    AccI
              Cfr10I   AvrII
                 —       —
GGCAGGGTGAGTTTTGAAGGATGCACCGGTCTACCTAGGACAGCCTATGTTTCTGATGAC   300

ACCCGATTCAAAGTGGGCACAGATGGTGTGATTACAGTCAAGCGGCCTCTACAACTTCAT   360

AAACCAGAGATAAGTTTCTTGTCCATGCCCTGGGACTCCAGCCGCAGGAAGCTCTCCACC   420

                                          BspHI
                                            —
AGAGTTAGGCTGAAGGCAGGCGACGCACCACCACCATCATGATGCTCCCTCTAAA        480

                    HgiAI
                      —
ACCCAGACAGAGGTGCTCACATTCCCAGTTCCCAGCATGGACTCAGAAGACAGAAGAGA    540

              PvuII            EaeI
                —                —
GACTGGGTTATCCCTCCTATCAGCTGCCCGGAAAACGAGAAAGGCCCATTCCTAAAAAC    600

                                              BalI
                                                —
CTGGTTCAGATCAAGTCTAACAGGGACAAAGAAATCAAGGTTTTCTACAGCATCACTGGC   660
```

FIG. 4r.

StyI
CAAGGAGCTGACGCCACCTCCTGTTGGTGTGTTTATTATTGAAAGAGAAACAGGATGGCTG 720

                                    BsmI
AAGGTGACTGAGCCTCTGGATAGAGAACAAATTGCTAAGTACATTCTCTACTCTCATGCC 780

                                              BclI
GTATCTTCTAAATGGGAATGCGGTTGAAGACCCAATGGAGATCGTGATCACGGTGACAGAT 840

                    AvaI   StyI   XhoII   BanI
CAGAATGACAACAAGCCCGAGTTCACCCAGGCAGTCTTCCAAGGATCTGTCACGGAAGGT 900

        BanI BspMI
GCCCCTTCCAGGCCACCTCTGTGATGCAGGTGACAGCCACAGATGCGGATGATGTGAAT 960

                                          HgiAI   BstXI
ACCTACAACGCTGCCATCGCTTACAGCATCCTCACACAAGACCCCCTCCTGCCTAGCAGC 1020

              StyI   BspMI
ATGATGTTCACTATCAACAAGGACACAGGAGTCATCAGCGTGCTCACCACTGGGCTGGAC 1080

CGAGAGGGTGTCCCCATGTACACCCTTGGTGGTTCAGGCTGCTGACCTGCAAGGCGAAGGC 1140

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FIG.4s.

```
                    Bcl I
                    PvuI I        AlwNI
                      |             |
TTAACTACAACTGCAACAGCTGTGATCAACAGTCACTGACACATCAATGATAACCCCCCATC  1200

                       BanI
                         |
TTCAACCCAACCACGTACCAGGGACGGGTGCCTGAGAACAAGGCTAACGTCGAAATCGCT  1260

                                         BglI
                                           |
GTACTCAAAGTGACGGATGCTGATGTCCCCGATACCCCGGCCTGGAGGGCTGTGTACACC  1320
         Bcl I
           |
ATATTGAACAATAACAATGATCAATTTGTTGTCACCACAGACCCAGTAACTAACGACGGC  1380

ATTTTGAAAACAACTAAGGGCTTGGATTTTGAGGACAAGCAGCAGTATGTCTTGTACGTG  1440
                                                   AlwNI
                                                     |
ACTGTGGTGAACGTGACCCCGTTTGAGGTCATCCTCTCCACCTCCACAGCCACTGTCACT  1500

GTGGACGTGGAAGATGTGAATGAAGCCCCCATCTTCATCCCTTGCCCAAAGGTAGTGTCA  1560
                                                   XhoI I
                                                   BamHI
                                                     |
ATCCCTGAAGACTTTGGTGTGTGGGGCCAGGAAATCACACATCCTACACCGCCGAGGATCCCAGAT  1620
                                                   Cfr10I
                                                     |
```

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FIG.4t.

ACATATATGGAACAGAGGATAACGTATCGGATTTGGAGGGATGCTGCCGGTTGGCTGGAG 1680

BanI
PflMI
AlwNI   AvaI CelII

GTTAATCCAGAATCTGGTGCCATTTCACTCGGGCTGGACAGAGAGGATTTTGAG 1740

HgiAI

CACGTGAAGAATAGCACGTATGAAGCCCTCATTATAGCCATTGACTTCGGTTCTCCAGTT 1800

GCTACTGGAACGGGAACTCTTCTACTGGTCCTCTCTGATGTGAATGACAATGGCCCCATT 1860

CCAGAACCTCGAAATATGGACTTCTGCCAGAAAAACCCACAGCCTCATGTCATCAACATC 1920

XhoII
BglII

ATTGATCCAGATCTTCCCCCCAACACATCTCCCTTCACAGCAGAACTAACACACGGCGCA 1980

HincII

AGTGTCAACTGGACCATCGAGTACAATGACCCAGCTCGTGAATCTCTAATTTGAAGCCA 2040

AAGAAAACTTTAGAGTTGGGTGACTACAAAATAAATCTCAAGCTCACAGATAACCAGAAC 2100

PvuII
HincII
BstEII

AAGGACCAGGTGACCACCCTATATGTGTTTGTGTGCGACTGCGAAGGTGTCGTCAACAGC 2160

FIG.4u.


```
                                                              BsmI
                                                               |
HaeII                                        BspMI
BbeI                                           |
NarI
AhaII
BanI
 ||
TGCAAGAGGACGGGCGCCTTACGCGCCGAAGCAGGCTTGCCAGGTTCCTGCCATCTTGGGCATT    2220

CTCGGAGGAATCCTCGCTCTACTAATCCTGATTCTCTGCTTCTCTGCTATTTGTTCGGAGG      2280

                                         SmaI
                                         XmaI
                                         AvaI
                  BanII                   |
                    |
AGAAGGGTGGTCAAAGAGCCCTTACTTCCCCAGAAGATGACACCCGGGACAATGTTTAT        2340

TACTATGATGAAGAAGGAGGTGGAGAGGATCAGGACTTTGAGCCAGTTGCAC              2400

EcoO109
EaeI
DraII
  |
AGGGGCCTGGATGCTCGGCCTGAAGTGACTCGCAATGATGGCCCCAACCCTCCTGAGT        2460

GTGCCCCAGTATCGGCCCCTGCCAATCCTGATGAAATTGGAAACTTTATTGATGAA          2520

AACCTGAAGGCAGCGGACACTGACCCCTACTGCTCCTCCTTATGACTTCTCTGCTCGTGTTT    2580
```

FIG. 4v.

```
                                                          SstI
                                                          SacI
                                                          HgiAI
                   XmnI                                   BanII
                    |                                       |
GACTATGAAGGAAGGCGGTTCTGAAGCTGCTAGTCTGCAGCTCCTGAACTCCCTCAGAGTCA   2640

GACCAAGACCAGGACTACCTGAATGAATGGGGCAATCGCTTCAAGAAGCTGGCG           2700

          NspHI                              NspHI
          AflIII                             AflIII
            |                                  |
GACATGTATGGGAGGTGGCGGAGGACGACTAGGGGGACTTGAGACAAATGAAGATGAGTCCTT  2760

ATACCATGTGGTAGAAAATGCCGAGGTGACTGTTTCAGCTCCCTTCATCTGAGAGGAAT     2820

TTCTGGAGAAGAGAAAAATGCACAGTGATATATAGTTAGGATAGTTAGGATTCTACTTTA    2880

 XhoII                                     HindIII
 BglII                                       |
   |
TAGATCTAATCTGTGTGTTGTTAGAACGATTTGACCTATTCTTTGAAGCTTTTTTTC       2940

 DraI                                       NspHI
 AhaIII                                     AflIII
   |                                          |
TTTCTTTCATCATTCTTTAAATGGTGATGCTGTCCAAAAGACCCCCACATGTTTATATT     3000

          EcoNI NheI
            |
TCAAAAGAATAGCTAAAGCCTCCAGAAGGTTCTGCTAGCAATTCGAGATTGCCTTATTG     3060
```

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FIG.4w.

```
                    DraI
                    AhaIII
                      |
ACTTGTCTCATTTTTTAAAGGAAGGTAGGGCTAAACTACCCTATTGTGTTGTGTGT   3120

GTGTGTGTATGTGTAATTATTTTTAATTGTGTTCTTTTTTCTCCTATCACTGCACTGGT   3180
                                          EcoNI
                                            |
GTCCCGTGTTCTAATAACCACTCCTTAACTCCTTCTGAACTTACATTGCCTCAGACAGGAG   3240

                BanII
                ApaI
                EcoO109
                DraII
          PstI  EaeI
           |     | |                            Tth111I
           |     | |                               |
TTCTCTGCTGCAGAAATTATTGGGCCCTTTCAGGATAAGAGACTTGGTCTTAGTTTGATG   3300

GTAGTGTGACTGGGTATTATGGACTCGTAAGGACTTAGTGGTTCTCCTTTTTATTTCC   3360

TAAGTACACATAAATTGAAATTCATATCCATCCACTGACTGTCTGCATTAAGTGTGTTTG   3420

      AatII
      AhaII
        |
TCATGTGGACGTCATTATTGGGCTACTTTGGTTCTGAACAAGGAGCATTGACCAGAAAAG   3480
                            BanI
                              |
GTGGTGAATTTTCAGGTGCCACTCAACTTCTAATGTTCACTTATCACTCAAACAGAAGAG   3540
```

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FIG.4x.

```
                                    PstI
                          PstI
                           |      |
TGATCTCTATTCTCGACGTTTAGCCGTAGTGCTCCTGCAGCTGCTGCAGCCAAAGATTGAAGGCCGGA    3600
         |
        StyI

TTGTCAAAGCCAAGGGCAACATGAAAAATGACTTGGAGGTGGCAGGCGGGATGGGTCAT    3660

                                                    SauI
                                                    Eco81I
                                           NruI    Bsu36I
                                             |        |
TGAGCCTGGCGTTTAGCAAACTGATGCTGAGGATAACTGAGGTGGCTCTACCTCTAGTC    3720

                                                 SauI
                                                 Eco81I
                                                 Bsu36I
                                                    |
CTGAAAATTCTGAAGAATGGAAGAATCCCGACAAGTGTGTCCTATCGCGATCCTTAGGTC    3780

                              BanI          AccI
                               |             |
ACAGTTGTACCTGAGGCCAAGAATCCCCAGGTGCCTGCTTTGTTAATGTCTACCGAAA    3840

            BanI                              SspI
             |                                 |
ATGCAGCCTGATCTGGACTCAGGTGCCCCAATTCTAAGTGTGCATAGAAAACTGACAATA    3900

       SauI
       Eco81I
       Bsu36I
          |
TTAGGAAATTCTTTTCCCCCCTTAGGGAGCAGGAAGAAAATATGACCCTAAAGGGTTTG    3960
```

SUBSTITUTE SHEET

GCAAAGGGAAGGTGGGGAGAGCTTTGACTTGGATTTTTTTAAATTGAAATGTGAACTTC　4020

DraI
AhaIII

AAGGAACTTTTGACAACCATGGGAAATAATTTATCTTAAATTGCTTTACTGTCTGTCAG　4080

StyI
NcoI

PvuII

CTGTTTTTCAAAGAAAAAAATCATCCCTGCAATCACTTCTTGGAATTGTCTTGATTT　4140

DraI
AhaIII

TTCAGCAATTTAAACTCTAATTTAGTCCCTGTATAGAGAATGTTAATGTTAGTTTTGAGTGT　4200

ATATGTGTGGGTACGGATAATTTTGTATTTCTTTAGGTCTCGGAAAAGGAAAAACAATT　4260

SspI

TAAGCTGCGAAAATTCTTAAATATTCATTTTTATAAATTTATTAAAGAATTTTGTTAAA　4320

AAAAAAAAAAA　4333

FIG. 4y.

FIG. 5.
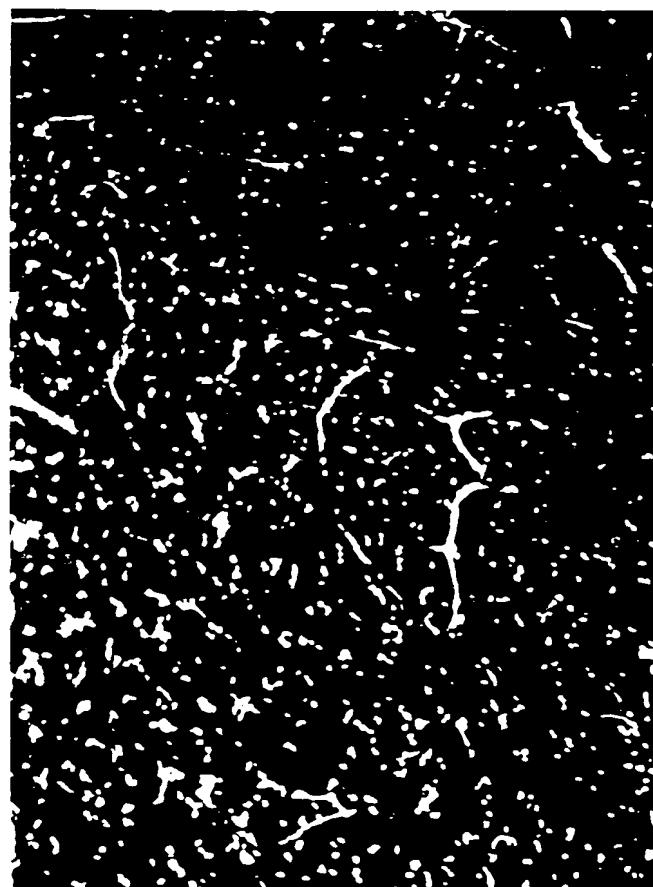
FIG. 6.
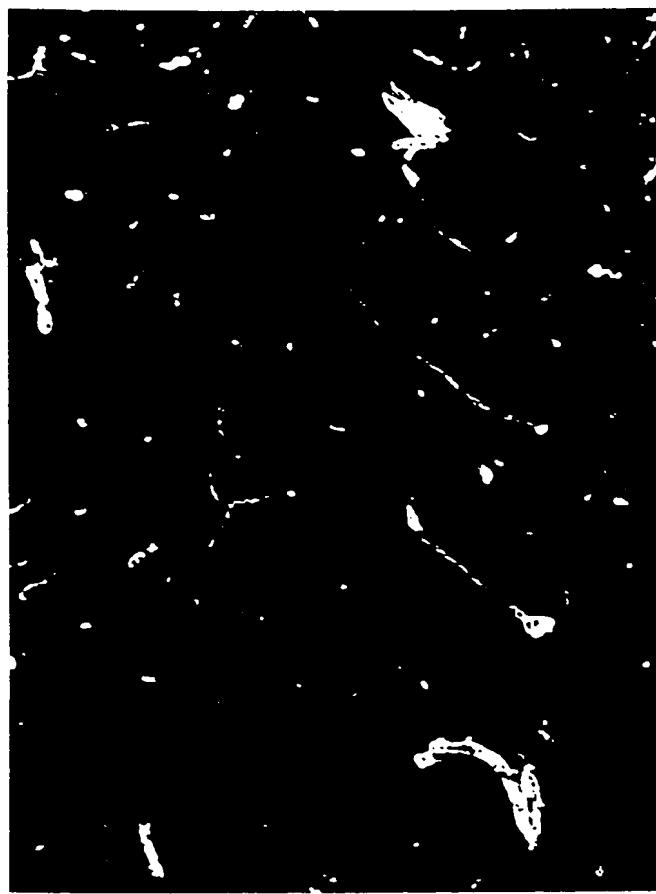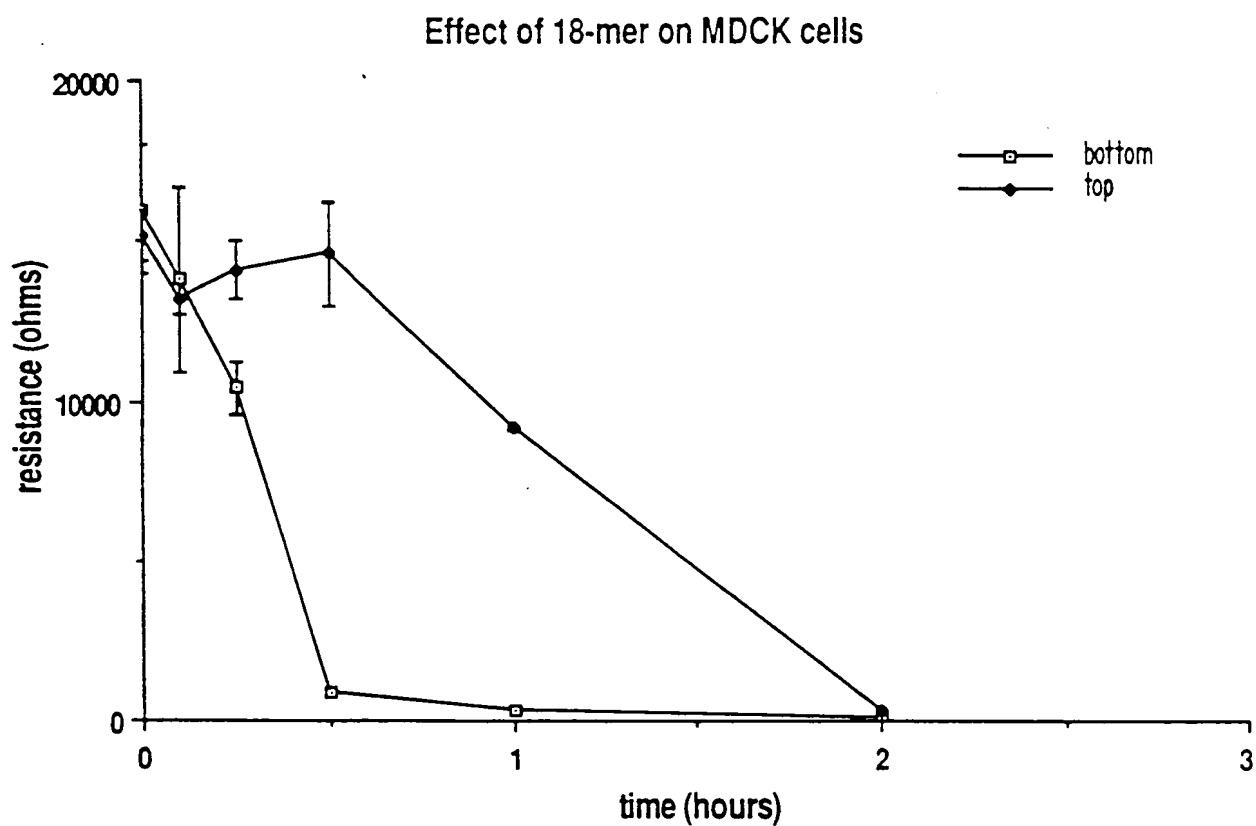
FIG. 7.
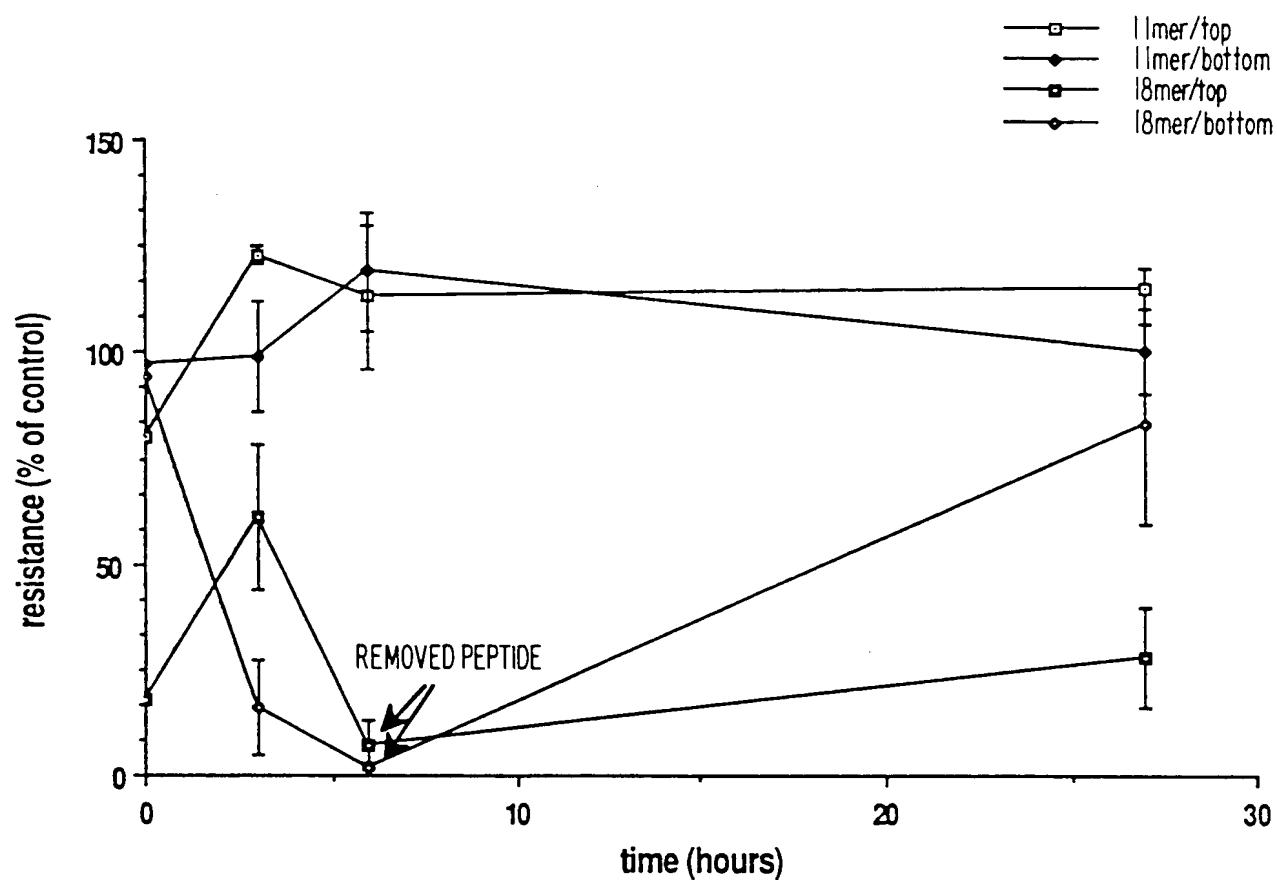
FIG. 8.
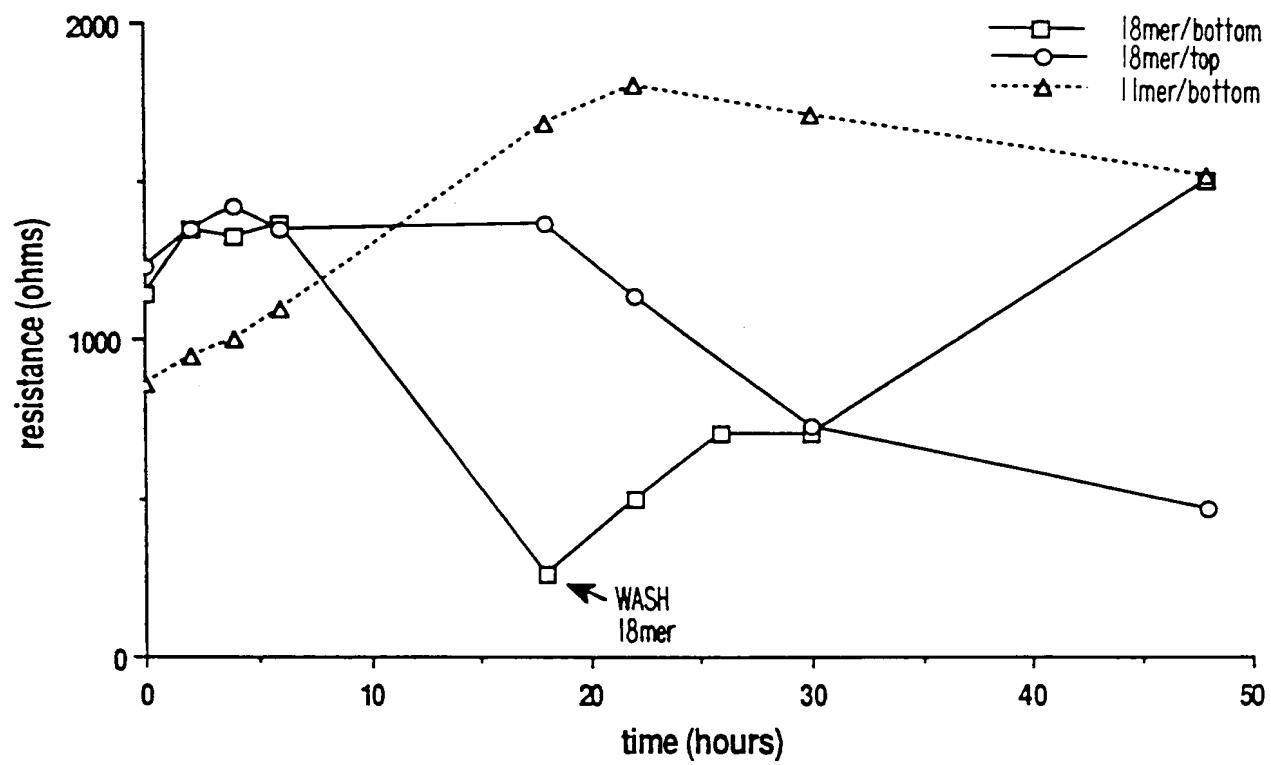
FIG. 9.

INTERNATIONAL SEARCH REPORT

International Application No PCT/US90/05105

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) ³

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC(5): A61K 37/02, 39/00; C07K 7/08. 7/10, 13/00, 15/00, 15/28
U.S.Cl.: 530/324, 326, 350, 389, 390, 391. 402, 409, 345, 387; 514/12, 13; 424/85.8, 85.91

II. FIELDS SEARCHED

Minimum Documentation Searched ⁴

Classification System	Classification Symbols
U.S. Cl.	530/324, 326, 350, 389, 390, 391, 402, 409, 345, 387 514/12, 13 424/85.8, 85.91

Documentation Searched other than Minimum Documentation
to the Extent that such Documents are Included in the Fields Searched ⁵

Data bases: Dialog (Files; Medline, Biosis, Chemical Abstracts, World Patents Index) Automated Patent Searching (1975-1990)

III. DOCUMENTS CONSIDERED TO BE RELEVANT ¹⁴

Category *	Citation of Document, ¹⁵ with indication, where appropriate, of the relevant passages ¹⁷	Relevant to Claim No. ¹⁸
X Y	The EMBO Journal, Volume 4, No. 13A, issued December 1985, Vestweben et al., "Identification of a Putative Cell Adhesion Domain of Uvomorulin," pp. 3393-3398. See the Abstract and Discussion.	1-6,14-21,23-27 & 35-42 1-65
Y	Development, Volume 102, issued April 1988, M. Takeichi, "The Cadherins: Cell-cell Adhesion Molecules controlling Animal Morphogenesis," pp. 639-655 see the Summary and pages 643, 645 and 651.	1-65
X Y	The Journal of Cell Biology, Volume 107, issued October 1988, B. Gumbiner et al., "The Role of the Cell Adhesion Molecule Uvomorulin in the Formation and Maintenance of the Epithelial Junctional Complex," pp. 1575-1587 see the Abstract.	1-6,14-21,23-27, 35-42 1-6,14-27,35-47, 55-65

* Special categories of cited documents: ¹⁵

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search ²

21 November 1990

Date of Mailing of this International Search Report ²

04 FEB 1991

International Searching Authority ¹

ISA/US

Signature of Authorized Officer ²⁰

R. Keith Baker, Ph.D.

Form PCT/ISA/210 (second sheet) (May 1986)

III. DOCUMENTS CONSIDERED TO BE RELEVANT   (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, '" with indication, where appropriate, of the relevant passages ''	Relevant to Claim No '*
Y	The EMBO Journal, Volume 6, No. 12, issued 1987, M. Ringwald et al., "The Structure of Cell Adhesion Molecule Uvomorulin Insights into the Molecular Mechanism of Ca^{--}-dependent Cell Adhesion," pp3347-3353, see pages 3647-3648.	1-13,22-34,43-54 and 63-65
Y	US, A, 4.671,958 (Rodwell et al.) 09 June 1987, see the Abstract and Column 7.	43-47 and 55-65
Y,P	Development Biology, Volume 139, No. 1, issued May 1990, O.W. Blaschuk et al., "Identification of a Cadherin Cell Adhesion Recognition Sequence," pp227-229, see the entire Document.	1-65

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers   . because they relate to subject matter [1] not required to be searched by this Authority, namely:

2. ☐ Claim numbers   . because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out [1], specifically:

3. ☐ Claim numbers _____, because they are dependent claims not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. X̄ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this international application as follows:

See Attachment

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.   Telephone Practice

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (supplemental sheet (2) (Rev. 4-90)

Attachment To PCT/ISA/210
Observations Where Unity Of Invention Is Lacking

Group I, claims 1-13 and 22-34, drawn to a composition for opening tight junctions and a method of use, classified in classes 530 and 514, subclasses 324, 326, 350 and 12 and 13, respectively.

Group II, claims 14-21 - 35-42, drawn to antibodies for opening tight junctions and methods of use, classified in classes 530 and 424, subclasses 387 and 85.8, respectively.

Group III, claims 43-54 and 63-65, drawn to a conjugates of a drug and a cell adhesion inhibitor, classified in class 530, subclasses 402, 409, and 345.

Group IV, claims 55-62, drawn to a conjugate of a drug and an antibody, classified in classes 530 and 424, subclasses 389, 390, 391 and 85.91, respectively.

Attachment To PCT/ISA/210
Detailed Reasons For Holding Lack Of Unity Of Invention:

PCT Rule 13.2 permits claims to "a" (one) product, "a" (one)
method of making and "a" (one) method of using said product. The
invention as set forth in Group I constitutes a combination of a
product and a method of use. Groups II, III and IV one drawn to
products that are distinct from that of Group I. Each of the
products have a different structure and are distinct compositions
as evidenced by their separate classification.
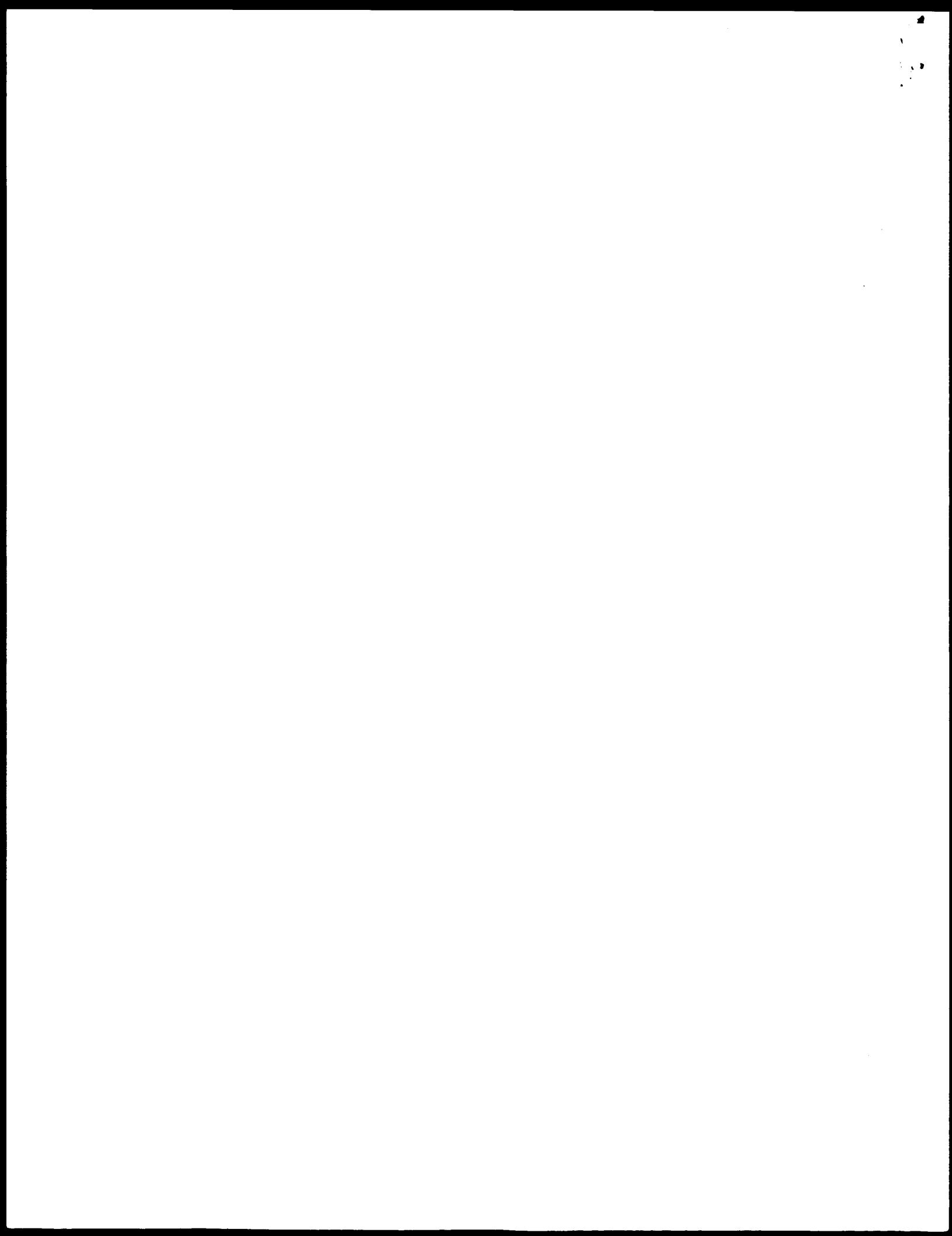
PN - JP4013631 - 920117
AP - JP900113456 900427
PA - TONEN CORP; others: 02
IN - UMEZAWA KAZUO; others: 02
I   - A61K35/78; C07G17/00
SI - C12N9/99
TI - SUBSTANCE ORGINATED FROM ANACARDIUM OCCIDENTALE
AB - NEW MATERIAL:A substance obtained from Anacardium occidentale and
      having physical chracteristics; thin layer chromatography: Rf=0.46
      (carrier silica gel, developing solvent; chloroform : methanol :
      concentrated ammonia water = 10 : 2 : 0.05); UV spectrum:
      lambdamax300nm (solvent: methanol); <1>H-NMR spectrum (solvent: heavy
      chloroform); deltappm: 0.9, 1.0-1.7, 2.0, 2.75, 5.0, 5.3, 5.8, 6.5,
      6.95, 7.5; solubility: soluble in hexane, chloroform, ethyl acetate,
      methanol and dimethylsulfoxide and isoluble in water, and having a
      tyrosine kinase-inhibiting activity and a beta-glucosidase-inhibiting
      activity.

Continue: Y / N
? Y
      - USE:An antitumor agent capable of simultaneously inhibiting or
        depressing the evolution and metastasis of cancers.
      - PREPARATION:The pericarps of Anacardium occidentale or seeds
        containing the pericarps are extracted with a solvent and the extract
        is purified with liquid chromatography.
GR - C0931
ABV - 016164
ABD - 920421
XPN - J04013631
XPR - 90JP-113456